



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US95/17082  <b>(22) International Filing Date:</b> 22 December 1995 (22.12.95)  <b>(30) Priority Data:</b> <table border="0"> <tr> <td>08/363,131</td> <td>23 December 1994 (23.12.94)</td> <td>US</td> </tr> <tr> <td>08/467,420</td> <td>6 June 1995 (06.06.95)</td> <td>US</td> </tr> <tr> <td>08/470,110</td> <td>6 June 1995 (06.06.95)</td> <td>US</td> </tr> </table> <b>(60) Parent Applications or Grants</b> <b>(63) Related by Continuation</b> <table border="0"> <tr> <td>US</td> <td>08/467,420 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>6 June 1995 (06.06.95)</td> </tr> <tr> <td>US</td> <td>08/470,110 (CIP)</td> </tr> <tr> <td>Filed on</td> <td>6 June 1995 (06.06.95)</td> </tr> </table> <b>(71) Applicants (for all designated States except US):</b> SMITHK-LINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW 2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). SMITHK-LINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> AMES, Robert. S. [US/US]; 276 Ellis Road, Havertown, PA 19083 (US). AP-PELBAUM, Edward, Robert [US/US]; 830 Village Circle, Blue Bell, PA 19422 (US). CHAIKEN, Irwin, M. [US/US]; 818 Youngsford Road, Gladwyn, PA 19035 (US). COOK,		08/363,131	23 December 1994 (23.12.94)	US	08/467,420	6 June 1995 (06.06.95)	US	08/470,110	6 June 1995 (06.06.95)	US	US	08/467,420 (CIP)	Filed on	6 June 1995 (06.06.95)	US	08/470,110 (CIP)	Filed on	6 June 1995 (06.06.95)	Richard, M. [GB/-]; 1006 Country Way, Chester Springs, PA 19425 (US). GROSS, Mitchell, Stuart [US/US]; 667 Pugh Road, Wayne, PA 19087 (US). HOLMES, Stephen, Dudley [GB/GB]; Yew Tree Bottom Road, Epsom, Surrey KT15 5XQ (GB). McMILLAN, Lynette, Jane [US/US]; 39 Wellington Road, Ardmore, PA 19003 (US). THEISEN, Timothy, Wayne [US/US]; 1902 Revolutionary Court, Phoenixville, PA 19460 (US).  <b>(74) Agents:</b> SUTTON, Jeffrey, A. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).  <b>(81) Designated States:</b> AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13<sup>bis</sup> separately from the description.</i> <i>Date of receipt by the International Bureau:</i> <b>11 March 1996 (11.03.96)</b>
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<b>(54) Title:</b> RECOMBINANT IL-5 ANTAGONISTS USEFUL IN TREATMENT OF IL-5 MEDIATED DISORDERS  <b>(57) Abstract</b>  Chimeric, humanized and other IL-5 mAbs, derived from high affinity neutralizing mAbs, pharmaceutical compositions containing same, methods of treatment and diagnostics are provided.																			

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RECOMBINANT IL-5 ANTAGONISTS USEFUL IN TREATMENT OF IL-5  
MEDIATED DISORDERS

Cross Reference to Related Applications

- 5           This application is a continuation-in-part of U.S. Serial Nos. 08/470,110 and 08/467,420, both filed June 6, 1995 which are continuation-in-parts of U.S. Serial No. 08/363,131 filed December 23, 1994.

Field of the Invention

- 10           The present invention relates generally to the field of antibodies and altered antibodies, useful in the treatment and diagnosis of conditions mediated by IL-5 and excess eosinophil production, and more specifically to mAbs, Fabs, chimeric and humanized antibodies.

15           Background of the Invention

- Eosinophils have been implicated in the pathogenesis of a wide variety of inflammatory disease states including allergic disorders associated with hypersensitivity reactions in the lung tissue (Butterfield et al., In: Immunopharmacology of Eosinophils, H. Smith and R. Cook, Eds., p.151-192, Academic Press, London (1993)). A notable example is asthma, a disease characterized by reversible obstruction of the airways leading to non-specific bronchial hyperresponsiveness. This in turn is dependent upon the generation of a chronic inflammatory reaction at the level of the bronchial mucosa and a characteristic infiltration by macrophages, lymphocytes and eosinophils. The eosinophil appears to play a central role in initiating the mucosal damage typical of the disease (Corrigan et al., Immunol. Today, 13:501-507 (1992)). Increased numbers of activated eosinophils have been reported in the circulation, bronchial secretions and lung parenchyma of patients with chronic asthma, and the severity of the disease, as measured by a variety of lung function tests, correlates with blood eosinophil numbers (Griffen et al., J. Aller. Clin. Immunol., 67:548-557 (1991)). Increased numbers of eosinophils, often in the process of degranulation, have also been recovered in bronchoalveolar lavage (BAL) fluids of patients undergoing late asthmatic reactions, and reducing eosinophil numbers, usually as a consequence of steroid therapy, is associated with improvements in clinical symptoms (Bousquet et al., N. Eng. J. Med., 323:1033-1039 (1990)).
- 35           Interleukin 5 (IL-5) is a homodimeric glycoprotein produced predominantly by activated CD4+ T lymphocytes. In man, IL-5 is largely responsible for controlling the growth and differentiation of eosinophils. Elevated levels of IL-5 are detected in the bronchoalveolar lavage washings of asthmatics (Motojima et al., Allergy, 48:98

(1993)). Mice which are transgenic for IL-5 show a marked eosinophilia in peripheral blood and tissues in the absence of antigenic stimulation (Dent et al., J. Exp. Med., 172:1425 (1990)) and anti-murine IL-5 monoclonal antibodies have been shown to have an effect in reducing eosinophilia in the blood and tissues of mice (Hitoshi et al., 5 Int. Immunol., 3:135 (1991)) as well as the eosinophilia associated with parasite infection and allergen challenge in experimental animals (Coffman et al., Science, 245:308-310 (1989), Sher et al., Proc. Natl. Acad. Sci., 83:61-65 (1990), Chand et al., Eur. J. Pharmacol., 211:121-123 (1992)).

Although corticosteroids are extremely effective in suppressing eosinophil 10 numbers and other inflammatory components of asthma, there are concerns about their side effects in both severe asthmatics and more recently in mild to moderate asthmatics. The only other major anti-inflammatory drug therapies - cromoglycates (cromolyn sodium and nedocromil) - are considerably less effective than corticosteroids and their precise mechanism of action remains unknown.

15 More recent developments have focused on new inhaled steroids, longer acting bronchodilators and agents acting on novel biochemical or pharmacological targets (e.g., potassium channel activators, leukotriene antagonists, 5-lipoxygenase (5-LO) inhibitors etc.). An ideal drug would be one that combines the efficacy of steroids with the safety associated with cromolyn sodium, yet has increased selectivity and 20 more rapid onset of action. Neutralizing IL-5 antibodies may potentially be useful in relieving eosinophila-related symptoms in man.

Hence there is a need in the art for a high affinity IL-5 antagonist, such as a neutralizing monoclonal antibody to human interleukin 5, which would reduce eosinophil differentiation and proliferation (i.e., accumulation of eosinophils) and thus 25 eosinophil inflammation.

### Summary of the Invention

In a first aspect, the present invention provides rodent (e.g., rat and murine) neutralizing monoclonal antibodies specific for human interleukin-5 and having a 30 binding affinity characterized by a dissociation constant equal to or less than about  $3.5 \times 10^{-11}$  M as described in the detailed description. Exemplary of such monoclonal antibodies are the murine monoclonal antibodies 2B6, 2E6 and 2F2 and rat monoclonal antibodies such as 4A6. Another aspect of the invention are hybridomas such as SK119-2B6.206.75(1), SK119-2E3.39.40.2, SK119-2F2.37.80.12, 4A6(1)G1F7 and 35 5D3(1)F5D6.

In a related aspect, the present invention provides neutralizing Fab fragments or  $F(ab')_2$  fragments thereof specific for human interleukin-5 produced by deleting the Fc region of the rodent neutralizing monoclonal antibodies of the present invention.

5 In yet another related aspect, the present invention provides neutralizing Fab fragments or  $F(ab')_2$  fragments thereof specific for human interleukin-5 produced by the chain shuffling technique whereby a heavy (or light) chain immunoglobulin, isolated from rodent neutralizing monoclonal antibodies of the invention, is expressed with a light chain (or heavy chain, respectively) immunoglobulin library isolated from interleukin-5 immunized rodents, in a filamentous phage Fab display library.

10 In still another related aspect, the present invention provides an altered antibody specific for human interleukin-5 which comprises complementarity determining regions (CDRs) derived from a non-human neutralizing monoclonal antibody (mAb) characterized by a dissociation constant equal to or less than about  $3.5 \times 10^{-11}$  M for human interleukin-5 and nucleic acid molecules encoding the same.

15 When the altered antibody is a humanized antibody, the sequences that encode complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner in which at least one, and preferably all complementarity determining regions (CDRs) of the first immunoglobulin partner are replaced by CDRs from the non-human monoclonal antibody. Preferably, the first

20 immunoglobulin partner is operatively linked to a second immunoglobulin partner as well, which comprises all or a part of an immunoglobulin constant chain.

In a related aspect, the present invention provides CDRs derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than about  $3.5 \times 10^{-11}$  M for human interleukin-5, and nucleic acid molecules encoding such CDRs.

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In still another aspect, there is provided a chimeric antibody containing human heavy and light chain constant regions and heavy and light chain variable regions derived from non-human neutralizing monoclonal antibodies characterized by a dissociation constant equal to or less than about  $3.5 \times 10^{-11}$  M for human interleukin-5.

30 In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) of the above-described altered antibodies and a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides a method for treating conditions in humans associated with excess eosinophil production by administering to

35 said human an effective amount of the pharmaceutical composition of the invention.

In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of altered antibodies (e.g.,

engineered antibodies, CDRs, Fab or F(ab)<sub>2</sub> fragments, or analogs thereof) which are derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than  $3.5 \times 10^{-11}$  M for human IL-5. These components include isolated nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that an altered antibody, preferably a humanized antibody, is expressed in said cells and isolating the expressed product therefrom.

In yet another aspect of the invention is a method to diagnose conditions associated with excess eosinophil production in a human which comprises obtaining a sample of biological fluid from a patient and allowing the antibodies and altered antibodies of the instant invention to come in contact with such sample under conditions such that an IL-5/(monoclonal or altered) antibody complex is formed and detecting the presence or absence of said IL-5/antibody complex.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

#### Brief Description of the Drawings

FIG. 1 [SEQ ID NOS: 1 and 15] illustrates the heavy chain variable region for the murine antibody 2B6, and the murine/human 2B6 chimeric antibody. The boxed areas indicate the CDRs.

FIG. 2 [SEQ ID NOS: 2 and 16] illustrates the light chain variable region for the murine antibody 2B6, and the murine/human 2B6 chimeric antibody. The boxed areas indicate the CDRs.

FIG. 3 [SEQ ID NO:3] illustrates the heavy chain variable region for the murine antibody 2F2. The boxed areas indicate the CDRs.

FIG. 4 [SEQ ID NO:4] illustrates the light chain variable region for the murine antibody 2F2. The boxed areas indicate the CDRs.

FIG. 5 [SEQ ID NO:5] illustrates the heavy chain variable region for the murine antibody 2E3. The boxed areas indicate the CDRs.

FIG. 6 [SEQ ID NO:6] illustrates the light chain variable region for the murine antibody 2E3. The boxed areas indicate the CDRs.

FIG. 7 [SEQ ID NOS:7-14] illustrates the heavy and light chain CDRs from murine antibodies 2B6, 2F2 and 2E3.

FIG. 8 [SEQ ID NOS: 18, 19] illustrates the heavy chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

FIG. 9 [SEQ ID NOS: 20, 21] illustrates the light chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

5        FIG. 10 is a schematic drawing of plasmid pCDIL5HZHC1.0 employed to express a humanized heavy chain gene in mammalian cells. The plasmid contains a beta lactamase gene (BETA LAC), an SV-40 origin of replication (SV40), a cytomegalovirus promoter sequence (CMV), a signal sequence, the humanized heavy chain, a poly A signal from bovine growth hormone (BGH), a betaglobin promoter (beta glopro), a dihydrofolate reductase gene (DHFR), and another BGH sequence poly A signal in a pUC19 background.

FIG. 11 is a schematic drawing of plasmid pCNIL5HZLC1.0 employed to express a humanized light chain gene in mammalian cells.

15        FIG. 12 [SEQ ID NOS: 61, 62] illustrates the NewM heavy chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

FIG. 13 [SEQ ID NOS: 69, 70] illustrates the REI light chain variable region for the humanized antibody 2B6. The boxed areas indicate the CDRs.

#### Detailed Description of the Invention

20        The present invention provides a variety of antibodies, altered antibodies and fragments thereof, which are characterized by human IL-5 binding specificity, neutralizing activity, and high affinity for human IL-5 as exemplified in murine monoclonal antibody 2B6. The antibodies of the present invention were prepared by conventional hybridoma techniques, phage display combinatorial libraries,  
25        immunoglobulin chain shuffling, and humanization techniques to generate novel neutralizing antibodies. These products are useful in therapeutic and pharmaceutical compositions for treating IL-5-mediated disorders, e.g., asthma. These products are also useful in the diagnosis of IL-5-mediated conditions by measurement (e.g., enzyme linked immunosorbent assay (ELISA)) of endogenous IL-5 levels in humans or IL-5  
30        released *ex vivo* from activated cells.

##### *I. Definitions.*

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies)  
35        or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab)<sub>2</sub> and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Neutralizing" refers to an antibody that inhibits IL-5 activity by preventing the binding of human IL-5 to its specific receptor or by inhibiting the signaling of IL-5 through its receptor, should binding occur. A mAb is neutralizing if it is 90% effective, preferably 95% effective and most preferably 100% effective in inhibiting IL-5 activity as measured in the B13 cell bioassay (IL-5 Neutralization assay, see Example 2C).

The term "high affinity" refers to an antibody having a binding affinity characterized by a  $K_d$  equal to or less than  $3.5 \times 10^{-11}$  M for human IL-5 as determined by optical biosensor analysis (see Example 2D).

By "binding specificity for human IL-5" is meant a high affinity for human, not murine, IL-5.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single



polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)<sub>2</sub> (i.e., a discrete part of an appropriate human constant region or framework region).

- 5 Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase,  $\beta$ -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab)<sub>2</sub> are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered

antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a non-human neutralizing monoclonal antibody (i.e., murine) designated as 2B6. The antibody 2B6 is defined as a high affinity, human-IL-5 specific (i.e., does not recognize murine IL-5), neutralizing antibody of isotype IgG<sub>1</sub> having the variable light chain DNA and amino acid sequences of SEQ ID NOs: 2 and 16, respectively, and the variable heavy chain DNA and amino acid sequences of SEQ ID NOs: 1 and 15, respectively, on a suitable murine IgG constant region.

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By 'sharing the antigen binding specificity or neutralizing ability' is meant, for example, that although mAb 2B6 may be characterized by a certain level of antigen affinity, a CDR encoded by a nucleic acid sequence of 2B6 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of 2B6 in such environments will nevertheless recognize the same epitope(s) as 2B6. Exemplary heavy chain CDRs of 2B6 include SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; and exemplary light chain CDRs of 2B6 include SEQ ID NO: 10; SEQ ID NO: 11; and SEQ ID NO: 12.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

5        An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be  
10        constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

      Analogues may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid or peptide sequences of the invention. Such variations or modifications may be due to  
15        degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

      The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor  
20        antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents  
25        may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol..

## *II. High Affinity IL-5 Monoclonal Antibodies*

      For use in constructing the antibodies, altered antibodies and fragments of this  
30        invention, a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) may be employed to generate a desirable immunoglobulin upon presentation with native human IL-5 or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to IL-5. Such hybridomas are then screened for binding  
35        using IL-5 coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated IL-5 bound to a streptavidin coated plate.

One exemplary, high affinity, neutralizing mAb of this instant invention is mAb 2B6, a murine antibody which can be used for the development of a chimeric or humanized antibody, described in more detail in Example 1 below. The 2B6 mAb is characterized by an antigen binding specificity for human IL-5, with a  $K_d$  of less than  $3.5 \times 10^{-11}$  M (about  $2.2 \times 10^{-11}$  M) for IL-5. The  $K_d$  for IL-5 of a Fab fragment from 2B6 (see, Example 3H) is estimated to be about  $9 \times 10^{-11}$  M as determined by optical biosensor. MAb 2B6 appears to block the binding interaction between human IL-5 and the  $\alpha$ -chain of the human IL-5 receptor.

Another desirable donor antibody is the murine mAb, 2E3. This mAb is characterized by being isotype IgG<sub>2a</sub>, and having a dissociation constant for hIL-5 of less than  $3.5 \times 10^{-11}$  M (about  $2.0 \times 10^{-11}$  M).

Yet, another desirable donor antibody is the rat mAb, 4A6. This mAb is characterized by having a dissociation constant for hIL-5 of less than  $3.5 \times 10^{-11}$  M (about  $1.8 \times 10^{-11}$  M). In addition, mAb 4A6 appears to block the binding interaction between human IL-5 and the  $\beta$ -chain of the IL-5 receptor.

This invention is not limited to the use of the 2B6 mAb, the 2E3 mAb, or its hypervariable (i.e., CDR) sequences. Any other appropriate high affinity IL-5 antibodies characterized by a dissociation constant equal or less than  $3.5 \times 10^{-11}$  M for human IL-5 and corresponding anti-IL-5 CDRs may be substituted therefor. Wherever in the following description the donor antibody is identified as 2B6 or 2E3, this designation is made for illustration and simplicity of description only.

### III. Antibody Fragments

The present invention also includes the use of Fab fragments or F(ab')<sub>2</sub> fragments derived from mAbs directed against human IL-5. These fragments are useful as agents protective *in vivo* against IL-5 and eosinophil-mediated conditions or *in vitro* as part of an IL-5 diagnostic. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab')<sub>2</sub> fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAbs 2B6, 2E3, and other similar high affinity, IL-5 binding antibodies, provide sources of Fab fragments and F(ab')<sub>2</sub> fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and F(ab')<sub>2</sub> fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')<sub>2</sub> fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via

immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety) wherein the Fd or  $V_H$  immunoglobulin from a selected antibody (e.g., 2B6) is allowed to associate with a repertoire of light chain immunoglobulins,  $V_L$  (or  $V_K$ ), to form novel Fabs.

- 5 Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins,  $V_H$  (or Fd), to form novel Fabs. Neutralizing IL-5 Fabs were obtained when the Fd of mAb 2B6 was allowed to associate with a repertoire of light chain immunoglobulins, as described in more detail in the Examples section. Hence, one is able to recover neutralizing Fabs  
10 with unique sequences (nucleotide and amino acid) from the chain shuffling technique.

#### *IV. Anti-IL-5 Amino Acid and Nucleotide Sequences of Interest*

- The mAb 2B6 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid  
15 sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

- As one example, the present invention thus provides variable light chain and variable heavy chain sequences from the IL-5 murine antibody 2B6 and sequences derived therefrom. The heavy chain variable region of 2B6 is illustrated by FIG. 1.  
20 The CDR-encoding regions are indicated by the boxed areas and are provided in SEQ ID NO: 7; SEQ ID NO: 8; and SEQ ID NO: 9. The light chain clone variable region of 2B6 is illustrated by FIG. 2. The CDR-encoding regions are provided in SEQ ID NO: 10; SEQ ID NO: 11; and SEQ ID NO: 12.

- A humanized heavy chain variable region is illustrated in Fig. 8 [SEQ ID NOs:  
25 18 and 19]. The signal sequence is also provided in SEQ ID NO: 17. Other suitable signal sequences, known to those of skill in the art, may be substituted for the signal sequences exemplified herein. The CDR amino acid sequences of this construct are identical to the native murine and chimeric heavy chain CDRs and are provided by SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9. An exemplary (synthetic)  
30 humanized light chain variable sequence is illustrated in Fig. 9 [SEQ ID NOs: 20 and 21].

- The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences encoding  
35 the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression. For example, silent substitutions in the nucleotide sequence of the framework and CDR-encoding regions

were used to create restriction enzyme sites which facilitated insertion of mutagenized CDR (and/or framework) regions. These CDR-encoding regions were used in the construction of a humanized antibody of this invention.

5 Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, 10 e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those 15 complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences. An 20 example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

25 V. *Altered immunoglobulin molecules and Altered antibodies*

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an IL-5 antibody, preferably a high 30 affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and 35 may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to which the light or heavy chain constant region is fused in

frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of IL-5 may be designed to elicit enhanced binding with the same antibody.

5 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

10 Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression. As one example the 2B6 humanized antibody having the signal sequence and CDRs derived from the murine heavy chain sequence, had the original signal peptide replaced with another signal sequence [SEQ ID NO: 17].

20 An exemplary altered antibody contains a variable heavy and/or light chain peptide or protein sequence having the antigen specificity of mAb 2B6, e.g., the  $V_H$  and  $V_L$  chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the murine antibody molecule 2B6 with the remaining sequences being derived from a human source, or a functional fragment or analog thereof. See, e.g., the humanized  $V_H$  and  $V_L$  regions (Figs. 8 and 9).

30 In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the  $F_c$  fragment or CH2 CH3 domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

35 The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of murine 2B6. The resulting protein may exhibit both anti-IL-5 antigen specificity and characteristics of the non-

immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

- 5           Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')<sub>2</sub> fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F<sub>v</sub> or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb 2B6 or 2E3.
- 10   Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

- Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies
- 15   can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor antibody, and one or more (preferably all) CDRs from the donor antibody, e.g., the anti-IL-5 antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or
- 20   the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

- Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the IL-5 mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs (see also FIG. 7).
- 25   The engineered antibodies of the invention are neutralizing, i.e., they desirably block binding to the receptor of the IL-5 protein and they also block or prevent proliferation of IL-5 dependent cells.

- Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric
- 30   antibody containing the human heavy and light chain constant regions fused to the IL-5 antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a
- 35   homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody



capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

One example of a particularly desirable humanized antibody contains CDRs of 2B6 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the IL-5 antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence, replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-5 mediated inflammatory diseases in man, or for diagnostic uses.

As another example, an engineered antibody may contain three CDRs of the variable light chain region of 2E3 [SEQ ID NO: 10, 11 and 13] and three CDRs of the variable heavy chain region of 2B6 [SEQ ID NO: 7, 8 and 9]. The resulting humanized antibody should be characterized by the same antigen binding specificity and high affinity of mAb 2B6.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is

anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization,  
5 binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol., 30:105-108 (1993), Xu et al., J. Biol. Chem., 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy  
10 chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies are useful in the prevention and treatment of IL-5 mediated  
15 disorders, as discussed below.

#### *VI. Production of Altered antibodies and Engineered Antibodies*

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb 2B6 or other suitable donor mAbs (e.g., 2E3, 2F2, 4A6, etc.), and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably  
20 humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the murine antibody 2B6, is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described  
25 in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual)), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions of 2B6 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the  
30 antibody chain derived from a human immunoglobulin are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor  
35 antibody V<sub>H</sub> and V<sub>L</sub> regions, in association with human Ig constant regions for both chains.

Homologous framework regions of a heavy chain variable region from a human antibody were identified using computerized databases, e.g., KABAT®, and a human antibody having homology to 2B6 was selected as the acceptor antibody. The sequences of synthetic heavy chain variable regions containing the 2B6 CDR-encoding regions within the human antibody frameworks were designed with optional nucleotide replacements in the framework regions to incorporate restriction sites. This designed sequence was then synthesized using long synthetic oligomers. Alternatively, the designed sequence can be synthesized by overlapping oligonucleotides, amplified by polymerase chain reaction (PCR), and corrected for errors.

A suitable light chain variable framework region was designed in a similar manner.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this technique on other suitable IL-5-specific, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional

techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or

altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

5           Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO, COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed.

10          The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

            Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev.,  
15    130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For  
20    example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

            Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and  
25    viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

            The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell  
30    are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention.

35           Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when

transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the engineered antibody to IL-5. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the procedures described for humanized antibodies prepared from 2B6, one of skill in the art may also construct humanized antibodies from other donor IL-5 antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies may effectively treat a human for IL-5 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

#### VII. Therapeutic/Prophylactic Uses

This invention also relates to a method of treating humans experiencing eosinophilia-related symptoms, such as asthma, which comprises administering an effective dose of antibodies including one or more of the engineered antibodies or altered antibodies described herein, or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by the binding to human IL-5 and thus subsequently blocking eosinophil stimulation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing an allergic and/or atopic response, or a response associated with eosinophilia, such as but not limited to, allergic rhinitis, asthma, chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis, coeliac disease, eosinophilic gastroenteritis, Churg-Strauss syndrome (periarteritis nodosa plus atopy), eosinophilic myalgia syndrome, hypereosinophilic syndrome, oedematous reactions including episodic angiodema, helminth infections, where eosinophils may have a protective role, onchocercal dermatitis and atopic dermatitis.

The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive

with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for treatment of allergic conditions from about 2 days to about 3 weeks, or as needed. For example, longer treatments may be desirable when treating seasonal rhinitis or the like. This represents a considerable advance over the currently used infusion protocol with prior art treatments of IL-5 mediated disorders. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical

composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.* or *i.m.* (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the inflammatory response.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-5 mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-5 levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

Thus, one embodiment of the present invention relates to a method for aiding the diagnosis of allergies and other conditions associated with excess eosinophil production in a patient which comprises the steps of determining the amount of human IL-5 in sample (plasma or tissue) obtained from said patient and comparing said determined amount to the mean amount of human IL-5 in the normal population, whereby the presence of a significantly elevated amount of IL-5 in the patient's sample is an indication of allergies and other conditions associated with excess eosinophil production.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this



invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T.

- 5 Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").

#### Example 1 - Production of MAbs to hIL-5

- 10 Human IL-5 was expressed in *Drosophila* Schneider 2 (S2) cells and purified to homogeneity. Murine IL-5 was expressed in Baculovirus using *Spodoptera frugiperda* 21 (Sf21) cells and purified to homogeneity. Monoclonal antibody TRFK-5 (a neutralizing rat anti-mouse IL-5 antibody) was obtained from Genzyme Corp. (Cambridge, MA).

##### *A. Immunization Procedure:*

- 15 Recombinant human IL-5 (IL-5) was used as the immunogen for a panel of seven CAF1 female mice (Charles River, Wilmington, MA). The animals received three subcutaneous injections of IL-5 in phosphate buffered saline (PBS) emulsified with a one to one ratio of TiterMAX™ (CytoRx Corp., Norcross, GA) over a period of four months. The priming antigen dose was 50 µg (micrograms) and boosts were 25  
20 and 10 µg (micrograms). After the boosts, serum samples were collected and assayed both for binding to IL-5 and for neutralization activity via the receptor binding inhibition assay and B13 proliferation assay (or IL-5 neutralization assay (Example 2C)). All of the mice produced serum samples that bound to IL-5. Animals selected as spleen donors were boosted intravenously with 10 µg (micrograms) of recombinant  
25 human IL-5 three days prior to euthanasia.

##### *B. Hybridoma Development:*

- The fusion procedure, first reported by Kohler et al., (Nature, 256:495 (1975)), was used with modifications to perform the technique using a cell monolayer (Kennet et al., Eds., "Hybridomas: A new dimension in biological analysis", pp. 368-377,  
30 Plenum Press, New York). Spleen cells from two donor mice were pooled and fusions performed using a ratio of 50 million spleen cells to ten million SP2/0/Ag14 myeloma cells. Supernatants from fusion-positive wells were assayed for binding to IL-5 by ELISA. Wells containing cells producing antibody to IL-5 were expanded and supernatants screened in an IL-5 receptor binding inhibition assay, and a B13  
35 (neutralization) proliferation assay (described below).

Sixteen hybridomas were isolated which secreted mAbs reactive with IL-5. The hybridoma supernatants were mixed with iodinated IL-5, added to a membrane

extract prepared from *Drosophila* cells expressing the  $\alpha$ -chain of the IL-5 receptor (IL-5R), and assayed for inhibition of receptor binding. Eleven of the hybridoma supernatants inhibited by greater than 60% the binding of iodinated IL-5 to the IL-5 receptor  $\alpha$ -chain. Three of the mAbs, 2B6, 2E3 and 2F2, also inhibited by greater than 70% the proliferation of murine B13 cells in response to human but not murine IL-5. Five of the hybridomas, four of which blocked binding and/or proliferation (1C6, 2B6, 2E3 and 2F2) and 1 of which was non-neutralizing (24G9), were repeatedly subcloned in soft agar to generate stable clonal cell lines. Supernatants from the cloned lines were screened for cross-reactivity by ELISA and did not bind to human IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-8, M-CSF or TGF $\alpha$ . The mAbs were purified and binding affinities were estimated from optical biosensor (BIAcore) analysis to range from 10 to 100 pM. Supernatants from the lines were isotyped using murine isotyping reagents (PharMingen, San Diego, CA). A summary of the affinities and IC<sub>50</sub> for neutralizing activities of the mAbs is presented in Table I (Example 2).

By similar methods, rat hybridomas were derived from immunized rats, using a comparable immunization protocol and rat myelomas for the fusion as described for the mouse. Two rat hybridomas, 4A6 and 5D3, were identified that produced mAbs which bound to IL-5. Like mAbs 2B6, 2E3 and 2F2, mAbs 4A6 and 5D3 were found to be neutralizing in the B13 assay described below.

#### C. Hybridoma Deposit:

The hybridoma cell line SK119-2B6.206.75(1) producing monoclonal antibody 2B6 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11783, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-2E3.39.40.2 producing monoclonal antibody 2E3 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11782, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-2F2.37.80.12 producing monoclonal antibody 2F2 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11781, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The hybridoma cell line SK119-24G9.8.20.5 producing monoclonal antibody 24G9 was deposited with the American Type Culture Collection (ATCC), Rockville,

MD, USA, under accession number HB 11780, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

5 The hybridoma cell line 4A6(1)G1F7 producing monoclonal antibody 4A6 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11943, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

10 The hybridoma cell line 5D3(1)F5D6 producing monoclonal antibody 5D3 was deposited with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number HB 11942, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

## 15 Example 2 - Assays

### A. ELISA:

Individual wells of MaxiSorb™ immuno plates (Nunc, Naperville, IL) were coated with 0.2 ug IL-5 in 0.05M carbonate buffer pH 9.6. After incubating overnight at 4°C, the plates were rinsed with PBS containing 0.025% Tween® 20, and blocked  
20 with 1% BSA in PBS with 0.025% Tween® 20 for two hours at room temperature. Undiluted hybrid supernatants were added to the IL-5 coated wells and incubated at room temperature for two hours. After the plates were rinsed, peroxidase labeled goat anti-mouse IgG & IgM (Boehringer Mannheim, Indianapolis, IN) was added at 1/7500 dilution in PBS containing 1% BSA and 0.025% Tween® 20. Two hours later the  
25 plates were washed and 0.2 ml of 0.1M citrate buffer pH 4.75 containing 0.1% urea peroxide and 1mg/ml orthophenylenediamine was added. After 15 min the plates were read at 450nm on a VMax™ Microplate Reader (Molecular Devices, Menlo Park, CA).

### B. Receptor Binding Inhibition Assay:

Membrane extracts of *Drosophila* S2 cells expressing the  $\alpha$ -chain of the human  
30 IL-5 Receptor (IL-5R) were used to measure the effect of antibody on IL-5 binding to receptor. To prepare the membranes,  $10^9$  cells were pelleted at 1000 x g at 4°C for 10 min. The cell pellet was frozen in a dry ice/ethanol bath for 15 min. The pellet was thawed, resuspended in 10 ml PBS at 4°C and pelleted at 1000 x g for 10 min. The cell pellet was washed 2X in PBS and resuspended in 13.5 ml Hypotonic buffer (10  
35 mM Tris pH 7.5, 3 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 1mM phenylmethylsulfonyl fluoride, 1 uM leupeptin, 1 uM pepstatin A) and incubated on ice for 5 min. The cell suspension was homogenized in a 15 ml Dounce homogenizer and brought to a final

concentration of 0.25 M sucrose with a solution of 2.5 M sucrose. Cell debris was removed by a 15 min centrifugation at 1000 x g. Cell membranes were pelleted at 100,000 x g at 4°C for 90 min and resuspended in 50 ml of 10 mM Tris pH 7.5, 3 mM MgCl<sub>2</sub>, 250 mM sucrose, and stored at -70°C.

- 5            Assays with *Drosophila* membranes containing receptor were performed in MultiscreenGV™ plates (Millipore Corp., Bedford, MA) using *Drosophila* tissue culture medium M3 (Lindquist et al., Drosophila Inf. Serv., 58: 163 (1982)) containing 25 mM HEPES buffer pH 7.2 and 0.1% BSA (Binding Buffer). Wells were pre-blocked with 0.1 ml binding buffer. 50 ul of the test sample, in triplicate, was added to  
10 wells followed by 25 ul iodinated (<sup>125</sup>I) IL-5. After 20 minutes incubation at room temperature, 25 ul of the membrane extract of *Drosophila* S2 cells expressing the α-chain of the human IL5R was added to the wells. After 1 hour further incubation the membranes were collected by vacuum filtration and washed 3X with binding buffer. Filters were dried and counted.

15    C. *IL-5 Neutralization Assay*:

- The murine IL-5/IL-3 dependent cell line LyH7.B13 (B13) was obtained courtesy of R. Palacios, Basel Institute of Immunology, Switzerland. Cells were subcultured twice weekly in RPMI 1640 medium (GibcoBRL, Renfrewshire, UK), supplemented with L-Glutamine, non-essential amino acids, sodium pyruvate,  
20 penicillin-streptomycin (all GibcoBRL), plus 2-mercaptoethanol ( $5 \times 10^{-5}$  M, Sigma), 10% fetal bovine serum (Globepharm, Surrey, UK ) and 1-10 units murine IL-5. For assays, cells were cultured for 48 hours in triplicate (5000 cells/well) in 96-well round bottom plates in the presence of appropriately diluted test samples and pulsed with 0.5 uCi <sup>3</sup>H-thymidine (Amersham, Bucks, UK ) for the final 4 hours. They were  
25 processed for scintillation counting in a 1205 Betaplate (LKB Wallac, Beds, UK).

          D. *Optical Biosensor*:

- Kinetic and equilibrium binding properties with immobilized hIL-5 and antibodies were measured using a BIAcore optical biosensor (Pharmacia Biosensor, Uppsala, Sweden). Kinetic data were evaluated using relationships described  
30 previously (Karlsson et al., J. Immunol. Meth., 145:229-240 (1991)) and which is incorporated by reference in its entirety.

- Three of the neutralizing mAbs, namely 2B6, 2E3 and 2F2, had very similar potencies of inhibition of <sup>125</sup>I-IL-5 binding to membrane receptor and neutralization  
35 of B cell proliferation and also very similar affinities for IL-5 (see Table I). The nucleotide sequences of the V<sub>H</sub> and V<sub>L</sub> from these three mAbs, 2 IgG1 and 1 IgG2a,

respectively, were determined. The sequences obtained were very similar, differing only at a few residues.

**TABLE I**  
Affinity and neutralizing activity of mAbs reactive with human IL-5

mAb	Kd (pM) <sup>a</sup>	Neutralization		
		Binding IC <sub>50</sub> (nM) <sup>b</sup>	Proliferation IC <sub>50</sub> <sup>c</sup>	100%Inhibition <sup>c</sup>
2B6	22	1	70	200
2E3	20	1	90	600
2F2	13	1	150	340
1C6	86	43	12,200	ND
24G9	ND	>133	>100,000	ND
4A6	18	>88	28	100
5D3	ND	ND	100	10,000

- 5 <sup>a</sup> Determined by optical biosensor (BIAcore) analysis (25°C)  
<sup>b</sup> Inhibition of <sup>125</sup>I-IL-5 binding to IL-5R(α chain) from *Drosophila* membranes  
<sup>c</sup> Inhibition of proliferation (in pM) of B13 cells in response to 8 pM human IL-5  
 ND = No data

10

### Example 3-Isolation and Characterization of IL-5 Fabs from Combinatorial Library

#### A. PCR and Combinatorial Library Construction:

RNA purified from the spleens of three mice was reverse transcribed with a cDNA kit (Boehringer Mannheim, Indianapolis, IN) using either the primer (dT)<sub>15</sub> supplied with the kit or the 3' Fd (IgG1, IgG2a & IgG3) and kappa light chain primers as described by Huse et al. (Science, 246:1275 (1989)) and Kang, S.A. (Methods: Companion Methods Enzymol., 2:111 (1991)) which are hereby incorporated by reference in their entirety. Immunoglobulin cDNAs were amplified by PCR using the primers and the thermal cycling conditions described (Huse et al. *supra*). The *Hot Start* technique using AmpliWax™ PCR Gem 100 (Perkin Elmer Cetus, Norwalk, CT) beads and the manufacturer's protocol was used for all of the reactions. The PCR products were gel purified, digested, and ligated into the pMKFabGene3 vector (Ames et al., J. Immunol., 152:4572 (1994)). The library titer following ligation with the Fd cDNAs was 5.1 X 10<sup>7</sup> CFU and following ligation with the kappa cDNAs was 1.5 X 10<sup>6</sup> CFU. XL1-Blue cells (Stratagene, La Jolla, CA) transformed with the phagemid library were infected with helper phage VCSM13 (Stratagene) and phage were prepared as described by Barbas and Lerner (Methods: Companion Methods Enzymol., 2:119 (1991)).

### B. *Biopanning*:

Four microtiter wells (Immulon II Removawell Strips, Dynatech Laboratories Inc., Chantilly, VA) were coated overnight at 4°C with IL-5 (1ug/well) in 0.1M bicarbonate, pH 8.6. The wells were washed with water and blocked with PBS containing 3% BSA at 37°C for 1 hour. The blocking solution was removed, and the library was added to microtiter wells (50 ul/well) and incubated at 37°C for 2 hours. Wells were washed 10 times with TBS/Tween® (50mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween® 20) and once with H<sub>2</sub>O prior to elution of the adherent phage with 0.1 M HCl, adjusted to pH 2.2 with glycine, containing 1 mg/ml BSA.

### 10 C. *Colony Lifts*:

Colony lifts from clones isolated from the third and fourth rounds of biopanning were processed as described (Barbas and Lerner, *supra*). Filters were incubated for 1 hour at room temperature with 0.5-1.0 uCi <sup>125</sup>I-IL-5, which had been iodinated using Bolton-Hunter reagent (NEN, Billerica, MA) following the manufacturers recommended procedure, in PBS containing 1% BSA, washed with PBS 0.25% Tween, and exposed to Kodak XAR film. Colonies expressing IL-5-reactive Fabs were detected by autoradiography.

### D. *Preparation of Soluble FABs*:

Phagemid DNAs were digested with NheI and SpeI to remove gene III and self-ligated. XL1-Blue cells were transformed, and isolated clones were grown overnight at 37°C in 5.0 ml super broth (SB) medium (30 g tryptone, 20 g yeast extract, 10 g 3-[N-Morpholino]propanesulfonic acid, MOPS with pH adjusted to 7) containing 1% glucose and 50 ug/ml carbenicillin. Cells from 1 ml of this culture were pelleted at 3500 rpm for 10 min in Beckman GS-6R centrifuge and used to inoculate 5 ml SB containing 50 ug/ml carbenicillin. Cultures were shaken for 1 hour at 37°C, Isopropyl-b-D-thiogalactopyranoside (IPTG; 1 mM) was added and the cultures were transferred to 28°C overnight. Soluble Fab was prepared from periplasmic extracts by lysing the cell pellet for 20 min at 4°C in 20% sucrose suspended in 30 mM Tris pH 8.0, followed by centrifugation in a Microfuge for 10 min. Fab concentrations were estimated by western blot by comparison to samples containing known amounts of murine Fab. The different bacterial periplasmic extracts contained similar concentrations of Fab, ranging from 1 to 20 ug/ml, as estimated by western blot analysis.

### E. *Purification of FABs*:

A chelating peptide was engineered onto the carboxy-terminal end of the heavy chain to aid in protein purification. Following removal of the M13 geneIII coding region, via digestion with NheI and SpeI, a pair of overlapping oligonucleotides: [SEQ ID NO: 43] 5'-CTAGCCACCACCACCACCACCTAA-3';

[SEQ ID NO: 44] 3'-GGTGGTGGTGGTGGTGGTGATTGATC-5'

encoding six histidine residues were subcloned into the Fab expression vector.

Induction of Fab expression was performed as described above. Following overnight induction at 28°C periplasmic lysate of the cell pellet was prepared by 30 min

- 5 incubation at 4°C in 20% sucrose, 30 mM TRIS pH 8.0. Urea and Brij-35 detergent were added to the clarified supernatant to final concentrations of 2M and 1% respectively. After stirring at room temperature for 1 hour, the treated and clarified supernatant was loaded at 0.5 ml/min directly onto a 5 ml Nickel-NTA metal chelating column (1.5 x 3 cm) equilibrated with buffer A (100 mM Na-Phosphate, 10 mM Tris, 10 0.3 M NaCl, 2 M urea, pH 8.0). After a 4 column volume (20 ml) wash bound materials were eluted with a 6 column volume (30 ml) reverse pH gradient from pH 8 to pH 4 in the same buffer as above. The purified Fabs eluted from the column in a sharp symmetrical peak at pH 5.5. They were >90% pure and free of DNA.

F. *FAB ELISA*:

- 15 Immulon II plates (Dynatech) were coated overnight at 4°C with protein suspended (1 mg/ml; 50 ml per well) in 0.1 M bicarbonate buffer, pH 8.6. Dilutions and washes were performed in PBS containing 0.05% Tween™ 20. Plates were washed and blocked for 1 hour with PBS containing 1% BSA at room temperature. Various dilutions of the bacterial supernatants containing soluble Fabs, or purified Fabs, were 20 added to the plates. Following a one hour incubation plates were washed and biotinylated goat anti-mouse kappa (Southern Biotechnology Associates, Inc., Birmingham, AL) was added (1:2000 dilution; 50 ul/well) for 1 hour. The plates were washed and streptavidin labeled horseradish peroxidase was added (1:2000 dilution; 50 ul/well) for 1 hour. The plates were washed, ABTS peroxidase substrate was added 25 (100 ul/well; Kirkegaard & Perry Laboratories, Gaithersburg, MD) and the optical density at 405 nm was read on a UVmax™ (Molecular Devices) microplate reader.

G. *Isolation and Characterization of Fabs from a Combinatorial Library*:

- Phage bearing Fabs to IL-5 were selected from the library by multiple rounds of biopanning against microtiter wells coated with IL-5. After 4 rounds of selection 30 IL-5 reactive Fabs were identified by a colony lift assay using <sup>125</sup>I-IL-5. Thirty four colonies from the third round and 4 colonies from the fourth round were identified which bound labeled IL-5. Binding to IL-5 was confirmed by direct binding ELISA using culture supernatants expressing the Fab-geneIII fusion protein. DNA was isolated from these colonies and, after removing the coding region of M13 gene III, 35 soluble Fab expression was induced. Periplasmic fractions were prepared and assayed by ELISA for binding to IL-5. The Fabs bound specifically to IL-5 with no demonstrable binding to another protein, rC5a.



The undiluted periplasmic extracts (containing 1 to 20 ug/ml Fab) were assayed in the IL-5R binding inhibition assay (Example 2). None of the Fabs inhibited binding of iodinated IL-5 to the IL-5R $\alpha$  by more than 35%.

H. *Conversion of Neutralizing mAb to a FAB:*

- 5        The Fd and  $\kappa$  cDNAs of mAb (2B6) were isolated by PCR using the conditions described above. The gel-purified fragments were subcloned into the pMKFabGene3 vector which had been modified to include the hexa-His sequence 3' of the gene III cDNA, resulting in the plasmid pMKFabGene3H. A functional, IL-5 binding Fab clone  
10        containing the 2B6 heavy and light chains was identified by a colony lift assay. Upon removal gene III via Nhe I/SpeI I digestion and self-ligation the heavy chain was fused in frame to the hexa-His, allowing purification as described above. In a dose dependent manner, this Fab inhibited receptor binding with an IC<sub>50</sub> of approximately 7.5 ug/ml, similar to that of the parent mAb, murine 2B6.

I. *Construction and Screening of Chain-Shuffled Library:*

- 15        The cDNA encoding the Fd of the neutralizing mAb 2B6 was subcloned as an XhoI/SpeI fragment into pMKFabGene3H which contained a SstI/XbaI fragment in lieu of a light chain cDNA. This phagemid was digested with SstI and XbaI and ligated with the SstI/XbaI digested light chain PCR product derived from the IL-5 immunized mice (described above). The library titer following ligation was  $4 \times 10^5$  CFU. Biopanning,  
20        and colony lift assay was performed as described above for the combinatorial library .

- The library was constructed by pairing the cDNA encoding the Fd of the neutralizing mAb 2B6 with the same light chain repertoire, recovered from the IL-5 immunized mice, used to generate the combinatorial library. This chain shuffled library was subjected to 4 rounds of biopanning vs immobilized IL-5 and the resultant  
25        colonies were assayed for IL-5 reactivity using the colony lift assay. Positive colonies, which bound iodinated IL-5, were further assayed by ELISA and the IL-5R $\alpha$  binding assay. Two of the Fabs, 2 & 15, recovered from the chain shuffled library blocked binding of IL-5 to the IL-5R $\alpha$  and inhibited IL-5 dependent proliferation in the B13 assay. The sequences of these 2 Vks were similar to the sequence of the 2B6 Vk, the  
30        original light chain partner for the 2B6 VH. The light chain sequences for Fab 2 & 15 are SEQ ID NOs: 45 and 46, respectively. For Fab 2, CDRs 1-3 are SEQ ID NOs: 10, 11 and 47, respectively. For Fab 15, CDRs 1-3 are SEQ ID NOs: 10, 11 and 48, respectively.

- 35        All antibody amino acid sequences listed below in Examples 4 and 5 use the KABAT numbering system which allows variability in CDR and framework lengths. That is, key amino acids are always assigned the same number regardless of the actual

number of amino acids preceding them. For example, the cysteine preceding CDR1 of all light chains is always KABAT position 23 and the tryptophan residue following CDR1 is always KABAT position 35 even though CDR1 may contain up to 17 amino acids.

5

#### Example 4 - Humanized Antibody

One humanized antibody was designed to contain murine CDRs within a human antibody framework. This humanized version of the IL-5 specific mouse antibody 2B6, was prepared by performing the following manipulations.

#### 10 A. *Gene Cloning*:

mRNA was isolated from each of the respective 2B6, 2F2 and 2E3 hybridoma cell lines (see Example 1) with a kit obtained from Boehringer Mannheim (Indianapolis, IN) and then reverse transcribed using the primer (dT)<sub>15</sub> supplied with a cDNA kit (Boehringer Mannheim) to make cDNA. PCR primers specific for mouse immunoglobulin were used to amplify DNA coding for domains extending from amino acid #9 (KABAT numbering system) of the heavy chain variable region to the hinge region and from amino acid #9 (KABAT numbering system) of the light chain variable region to the end of the constant region. Several clones of each antibody chain were obtained by independent PCR reactions.

20 The mouse gamma 1 hinge region primer used is [SEQ ID NO: 22]:

5' GTACATATGCAAGGCTTACAACCACAATC 3'.

The mouse gamma 2a hinge region primer used is [SEQ ID NO: 23]:

5' GGACAGGGCTTACTAGTGGGCCCTCTGGGCTC 3'

The mouse heavy chain variable region primer used is [SEQ ID NO: 24]:

25 5' AGGT(C or G)(C or A)A(G or A)CT(G or T)TCTCGAGTC(T or A)GG 3'

The mouse kappa chain constant region primer used is [SEQ ID NO: 25]:

5' CTAACACTCATTCTGTTGAAGCTCTTGACAATGGG 3'

The mouse light chain variable region primer is [SEQ ID NO: 26]:

30 5' CCAGATGTGAGCTCGTGATGACCCAGACTCCA 3'

The PCR fragments were cloned into plasmids pGEM7f+ (Promega) that were then transformed into *E. coli* DH5a (Bethesda Research Labs).

#### B. *DNA Sequencing*:

35 The heavy and light chain murine cDNA clones from Part A above were sequenced. The results of sequencing of the variable regions of these clones are shown in SEQ ID NOs:1-6 (Fig. 1-6). Each clone contained amino acids known to

be conserved among mouse heavy chain variable regions or light chain variable regions. The CDR amino acid sequences are listed below.

The CDR regions for the 2B6 heavy chain are SEQ ID NOs: 7, 8 and 9. See Fig. 7. These sequences are encoded by SEQ ID NO:1. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 12. See Fig. 7. These sequences are encoded by SEQ ID NO:2.

The CDR regions for the 2F2 heavy chain are SEQ ID NOs: 7, 8 and 9. See Fig. 7. These sequences are encoded by SEQ ID NO:3. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 13. See Fig. 7. These sequences are encoded by SEQ ID NO:4.

The CDR regions for the 2E3 heavy chain are SEQ ID NOs: 7, 8 and 14. See Fig. 7. These sequences are encoded by SEQ ID NO:5. The CDR regions for the light chain are SEQ ID NOs: 10, 11 and 13. See Fig. 7. These sequences are encoded by SEQ ID NO:6.

#### C. Selection of Human Frameworks:

Following the cloning of 2B6, the amino acid sequences of the variable region heavy and light chains (Figs. 1 and 2) (SEQ ID NOs: 15 and 16, respectively) were compared with the known murine immunoglobulin sequences in the KABAT and SWISS-PROT (Nuc. Acids Res., 20:2019-2022 (1992)) protein sequence databases in order to assign amino acids to the N-terminal residues. The 2B6 heavy and light chain variable region deduced amino acid sequences were then compared with the human immunoglobulin protein sequence databases in order to identify a human framework for both the heavy and light chains which would most closely match the murine sequence. In addition, the heavy and light chains were evaluated with a positional database generated from structural models of the Fab domain to assess potential conflicts due to amino acids which might influence CDR presentation. Conflicts were resolved during synthesis of the humanized variable region frameworks by substitution of the corresponding mouse amino acid at that location.

The heavy chain framework regions of an antibody obtained from a human myeloma immunoglobulin (COR) was used (E. M. Press and N. M. Hogg, Biochem. J., 117:641-660 (1970)). The human heavy chain framework amino acid sequence was found to be approximately 66% homologous to the 2B6 framework.

For a suitable light chain variable region framework, the light chain variable framework sequence of the Bence-Jones protein, (LEN) (Schneider et al., Hoppe-Seyler's Z. Physiol. Chem., 356:507-557 (1975)), was used. The human

light chain framework regions were approximately 82% homologous to the murine 2B6 light chain framework regions, at the amino acid level.

The selected human frameworks were back translated to provide a DNA sequence.

5 D. *Construction of Humanized MAb Genes:*

Given the 2B6 heavy chain CDRs [Fig. 7 and SEQ ID NOs: 1-2] and the framework sequences of the human antibodies, a synthetic heavy chain variable region was made [SEQ ID NO: 18]. This was made using four synthetic oligonucleotides [SEQ ID NOs: 27 and 28] [SEQ ID NOs: 29 and 30] which, when  
10 joined, coded for amino acids #21-#106 (KABAT numeration). The oligonucleotides were then ligated into the HpaI-KpnI restriction sites of a pUC18 based plasmid containing sequences derived from another humanized heavy chain based on the COR framework (*supra*). This plasmid provides a signal sequence [SEQ ID NO: 17] and the remaining variable region sequence. Any errors in the  
15 mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites.

The signal sequence and humanized heavy chain variable region were excised from the pUC based plasmid as a EcoRI-ApaI fragment and ligated into the expression vector pCD that contained an IgG<sub>1</sub> human constant region. The  
20 synthetic heavy chain variable region nucleotide and amino acid sequences are provided in Fig. 8 [SEQ ID NOs: 18 and 19]. The human framework residues are amino acids 1-30, 36-49, 66-97 and 109-119 of SEQ ID NO: 19. The amino acid sequences of the CDRs are identical to the murine 2B6 CDRs. The resulting expression vector, pCDIL5HZHC1.0, is shown in Fig. 10.

25 Given the 2B6 light chain CDRs [Fig. 7 and SEQ ID NOs: 10, 11 and 12] and the framework sequence of the human antibody, a synthetic light chain variable region was made [SEQ ID NO: 20]. Four synthetic oligonucleotides coding for amino acids #27-#58 (KABAT numeration) [SEQ ID NOs: 31 and 32] and amino acids #80-#109 [SEQ ID NOs: 33 and 34] of the humanized V<sub>L</sub> with  
30 SacI-KpnI and PstI-HindIII ends respectively, were inserted into a pUC18 based plasmid containing sequences derived from another human light chain framework (B17) (Marsh et al, Nuc. Acids Res., 13:6531-6544 (1985)) which shares a high degree of homology to the LEN framework. This plasmid provides the remaining variable region sequence. Any errors in the mapped sequence and the single amino  
35 acid difference between the LEN and B17 frameworks were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites.

The humanized light chain variable region was isolated from the pUC plasmid as a EcoRV-NarI fragment and ligated into the expression vector pCN that contained a signal sequence [SEQ ID NO: 17] along with a kappa human constant region. The synthetic light chain variable region nucleotide and amino acid sequences are provided in Fig. 9 [SEQ ID NOs:20 and 21]. The human framework residues are amino acids 1-23, 41-55, 63-94 and 104-113 of SEQ ID NO: 21. The amino acid sequences of the CDRs are identical to the murine 2B6 CDRs. However, the coding sequences for these CDRs differ from the murine 2B6 coding sequences to allow creation of restriction enzyme sites. One of the resulting expression vectors, pCNIL5HZLC1.0, is shown in Fig. 11. These synthetic variable light and/or heavy chain sequences are employed in the construction of a humanized antibody.

*E. Expression of Humanized MAb:*

The humanized heavy chain, derived from an IgG<sub>1</sub> isotype, utilizes a synthetic heavy chain variable region as provided in SEQ ID NO:19. This synthetic V<sub>H</sub> containing the 2B6 heavy chain CDRs was designed and synthesized as described above.

The humanized light chain, a human kappa chain, utilizes a synthetic light chain variable region as provided in SEQ ID NO: 21. This synthetic V<sub>L</sub> containing the 2B6 light chain CDRs was designed and synthesized as described above. The DNA fragments coding for the humanized variable regions were inserted into pUC19-based mammalian cell expression plasmids that utilize a signal sequence and contain CMV promoters and the human heavy chain or human light chain constant regions of the chimera produced in Example 5 below, by conventional methods (Maniatis *et al.*, cited above) to yield the plasmids pCDIL5HZHC1.0 (heavy chain) [SEQ ID NO: 49, see also FIG. 10] and pCNIL5HZLC1.0 (light chain) [SEQ ID NO: 50, see also FIG. 11]. The plasmids were co-transfected into COS cells and supernatants assayed after three and five days, respectively, by the ELISA described in Example 5 for the presence of human antibody.

The above example describes the preparation of an exemplary engineered antibody. Similar procedures may be followed for the development of other engineered antibodies, using other anti-IL-5 antibodies (e.g., 2F2, 2E3, 4A6, 5D3, 24G9, etc.) developed by conventional means.

*F. Purification:*

Purification of CHO expressed chimeric and humanized 2B6 can be achieved by conventional protein A (or G) affinity chromatography followed by ion exchange and molecular sieve chromatography. Similar processes have been successfully

employed for the purification to >95% purity of other mAbs (e.g., to respiratory syncytial virus, interleukin-4 and malaria circumsporozoite antigens).

*G. Additional Humanized mAbs and Expression Plasmids:*

Given the plasmid pCDIL5HZHC1.0 [SEQ ID NO: 49] the expression plasmid  
5 pCDIL5HZHC1.1 was made that substitutes an Asparagine for Threonine at  
framework position 73. This was done by ligating a synthetic linker with EcoRV and  
XhoI ends [SEQ ID NO: 51 and SEQ ID NO: 52] into identically digested  
pCDIL5HZHC1.0. Similarly, the expression plasmid pCDIL5HZHC1.2 substitutes an  
Isoleucine for Valine at framework position 37. This was accomplished by ligating a  
10 synthetic linker with HpaI and XbaI ends [SEQ ID NO: 53 and SEQ ID NO: 54] into  
identically digested pCDIL5HZHC1.0. The expression plasmid pCDIL5HZHC1.3 was  
also made by ligating a synthetic linker with HpaI and XbaI ends [SEQ ID NO: 53 and  
SEQ ID NO: 54] into identically digested pCDIL5HZHC1.1.

Given the pUC18 based plasmid described previously which contains DNA  
15 sequences of four synthetic oligonucleotides [SEQ ID NOs: 31, 32, 33 and 34], a  
humanized light chain variable region was made where framework position #15 is  
changed from a Leucine to Alanine. This plasmid was digested with NheI and SacI  
restriction endonucleases and a synthetic linker [SEQ ID NOs: 55 and 56] was  
inserted. An EcoRV-NarI fragment was then isolated and ligated into the identically  
20 digested expression vector pCNIL5HZLC1.0 to create pCNIL5HZLC1.1.

A synthetic variable region was made using the heavy chain framework regions  
obtained from immunoglobulin (NEW) (Saul et al, J. Biol. Chem. 253:585-597(1978))  
and the 2B6 heavy chain CDRs [Fig. 7 and SEQ ID NOs: 1-2]. Framework amino  
acids which might influence CDR presentation were identified and substitutions made  
25 using methods described previously. Four overlapping synthetic oligonucleotides were  
generated [SEQ ID NOs: 57, 58, 59 and 60] which, when annealed and extended, code  
for amino acids representing a signal sequence [SEQ ID NO: 17] and a heavy chain  
variable region. This synthetic gene was then amplified using PCR primers [SEQ ID  
NOs: 63 and 64] and ligated as a BstXI-HindIII restriction fragment into a pUC18  
30 based plasmid containing sequences derived from another humanized heavy chain  
based on the COR framework. A phenylalanine to tyrosine framework substitution  
was made at amino acid position 91 (Kabat numbering system) (equivalent to position  
94 of Figure 12) by inserting a synthetic oligonucleotide linker [SEQ ID NOs: 75 and  
76] into SacII and KpnI restriction sites. The resulting heavy chain variable region  
35 [Fig. 12 and SEQ ID NOs: 61, 62] is referred to as the NEWM humanized heavy  
chain.

Any errors in the mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites. The signal sequence and humanized heavy chain variable region were excised from the pUC based plasmid as a EcoRI-ApaI fragment and ligated into the expression vector pCD that contained a human IgG<sub>1</sub> constant region to create the plasmid pCDIL5NEWM. The amino acid sequences of the CDRs are identical to the murine 2B6 heavy chain CDRs.

A synthetic variable region was made using the light chain framework regions obtained from immunoglobulin (REI) (Palm et al, Hoppe-Seyler's Z. Physiol. Chem. 356:167-191(1975)) and the 2B6 light chain CDRs [Fig. 7 and SEQ ID NOs: 10, 11 and 12]. Framework amino acids which might influence CDR presentation were identified and substitutions made using methods described previously. Four overlapping synthetic oligonucleotides were generated [SEQ ID NOs: 65, 66, 67 and 68] which, when annealed and extended, code for amino acids representing a light chain variable region [Fig. 13 and SEQ ID NOs: 69, 70] referred to as the REI humanized light chain. This synthetic gene was then amplified using PCR primers [SEQ ID NOs: 71 and 72] and ligated as an EcoRI-HindIII restriction fragment into pGEM-7Zf(+) (Promega Corporation, Madison, WI).

Any errors in the mapped sequence were corrected by PCR with mutagenic primers or by the addition of synthetic linkers into existing restriction sites. The humanized light chain variable region was excised from the pGEM-7Zf(+) based plasmid as an EcoRV-NarI fragment and ligated into the expression vector pCN that contained a signal sequence [SEQ ID NO: 17] along with a human Kappa constant region to create the plasmid pCNIL5REI. The amino acid sequences of the CDRs are identical to the murine 2B6 light chain CDRs. However, the coding sequences for these CDRs differ from the murine 2B6 coding sequences to allow creation of restriction enzyme sites. These synthetic variable light and/or heavy chain sequences are employed in the construction of a humanized antibody.

Given the pGEM-7Zf(+) based plasmid described above, a humanized light chain variable region can be made where framework position #15 is changed from a Valine to Alanine. This plasmid may be digested with NheI and SacI restriction endonucleases and a synthetic linker [SEQ ID NOs: 73 and 74] is inserted. An EcoRV-NarI fragment may then be isolated and ligated into the identically digested expression vector pCNIL5HZREI to create the plasmid pCNIL5REIV15A.

### Example 5 - Construction of a Chimeric Antibody

DNA coding for amino acids #9-#104 (KABAT numeration) of the murine mAb 2B6 heavy chain variable region was isolated as a *AvaII*-*StyI* restriction  
5 fragment from a pGEM7Zf+ based PCR clone of cDNA generated from the 2B6 hybridoma cell line (see Example 4). The flanking heavy chain variable region sequences and a signal sequence [SEQ ID NO: 17] were provided by combining this fragment along with four small synthetic oligomer linkers [SEQ ID NOs: 35 and 36] [SEQ ID NOs: 37 and 38] into a pUC18 based plasmid digested with  
10 *BstX1*-*HindIII*. A consensus of N-terminal amino acids deduced from closely related murine heavy chains were assigned for the first eight V<sub>H</sub> residues and are coded within SEQ ID NOs: 35 and 36. The deduced amino acid sequence of the heavy chain was verified by the sequencing of the first 15 N-terminal amino acids of the 2B6 heavy chain.

15 An *EcoRI*-*ApaI* fragment containing sequence for signal and V<sub>H</sub> regions was isolated and ligated into plasmid pCD that already encodes the human IgG1 constant region.

DNA coding for amino acids #12-#99 (KABAT nomenclature) of the murine mAb 2B6 light chain variable region was isolated as a *DdeI*-*AvaI*  
20 restriction fragment from a pGEM7Zf+ based PCR clone of cDNA generated from the 2B6 hybridoma cell line (see Example 4). The flanking light chain variable region sequences were provided by combining this fragment along with four small synthetic oligomer linkers [SEQ ID NOs: 39 and 40] [SEQ ID NOs: 41 and 42] into a pUC18 based plasmid digested with *EcoRV*-*HindIII*. A consensus of N-  
25 terminal amino acids deduced from closely related murine light chains were assigned for the first eight V<sub>L</sub> residues and are coded within SEQ ID NOs: 39 and 40. The deduced amino acid sequence of the light chain was verified by the sequencing of the first 15 N-terminal amino acids of the 2B6 light chain. This variable region was then isolated as a *EcoRV*-*NarI* fragment and ligated into the  
30 expression vector pCN that already contains the human kappa region and a signal sequence.

Expression of a chimeric antibody was accomplished by co-transfection of the pCD and pCN based plasmids into COS cells. Culture supernatants were collected three and five days later and assayed for immunoglobulin expression by  
35 ELISA described as follows: Each step except for the last is followed by PBS washes. Microtiter plates were coated overnight with 100 ng/50 ul/well of a goat antibody specific for the Fc region of human antibodies. The culture supernatants



were added and incubated for 1 hour. Horseradish peroxidase conjugated goat anti-human IgG antibody was then added and allowed to incubate for 1 hour. This was followed by addition of ABTS peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). After 1 hour incubation, the absorbance at 405 nm was read on a microtiter plate reader (Molecular Devices Corporation, Menlo Park, CA). Expression of the chimeric antibody was detected. In a similar ELISA, the COS cell supernatants, containing the chimeric antibody, bound specifically to microtiter wells coated with human IL-5 protein. This result confirmed that genes coding for an antibody to IL-5 had been synthesized and expressed.

The above example describes the preparation of an exemplary engineered antibody. Similar procedures may be followed for the development of other engineered antibodies, using other anti-IL-5 donor antibodies (e.g., 2F2, 2E3, 4A6, 5D3, 24G9, etc.) developed by conventional means.

15

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

- (i) APPLICANT: Ames, Robert S.  
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- (ii) TITLE OF INVENTION: Recombinant IL5 Antagonists Useful in  
Treatment of IL5 Mediated Disorders

- (iii) NUMBER OF SEQUENCES: 76

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- (A) MEDIUM TYPE: Floppy disk  
30 (B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

35

- (A) APPLICATION NUMBER:  
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5

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10

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## (2) INFORMATION FOR SEQ ID NO:1:

25

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 334 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

35

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..334

(D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat position 24"

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACTGTCT CTGGGTTTTC 60  
ATTAACCAGC TATAGTG TAC ACTGGGTTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120  
10 GGGAGTAATA TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180  
CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCAAACTGA 240  
15 TGACACAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTCT TCCTTACTAC GGCTTGACTA 300  
CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

(2) INFORMATION FOR SEQ ID NO:2:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 315 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: DNA (genomic)

30

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..315
- (D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat position 25"

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCCTCCCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT 60  
CTGTTAACA GTGGAAATCA AAAGAACTAC TTGGCCTGGT ACCAGCAGAA ACCAGGGCAG 120  
5 CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC 180  
ACAGGCAGTG GATCTGGAAC CGATTTCACT CTTTCCATCA GCAGTGTGCA GGCTGAAGAC 240  
10 CTGGCAGTTT ATTACTGTCA GAATGTTCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA 300  
GAGTTGGAAA TAAAA 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 334 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..334

(D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat position 24"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACTGTCT CTGGGTTTTTC 60  
35 ATTAACCACT TATAGTGTAC ACTGGGTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120

GGGAGTAATA TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180  
CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCGAAGTGA 240  
5 TGACACAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTCT TCCTTACTAC GGCTTGACTA 300  
CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

(2) INFORMATION FOR SEQ ID NO:4:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 315 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..315

(D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat 25"

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCTCCCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT 60  
30 CTATTAAACA GTGGAAATCA AAAGAACTAC TTGGCCTGGT ACCAACAGAA ACCAGGGCAG 120  
CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC 180  
35 ACAGGCAGTG GATCTGGAAC CGATTTCCT CTTACCATCA GCAGTGTGCA GGCTGAAGAC 240  
CTGGCAGTTT ATTACTGTCA GAATGATCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA 300

GAGTTGGAAA TAAAA

315

(2) INFORMATION FOR SEQ ID NO:5:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 334 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(ix) FEATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION: 1..334

(D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat position 24"

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCTGGCCTG GTGGCGCCCT CACAGAGCCT GTCCATCACT TGCACGTGTCT CTGGGTTTTTC 60

25

ATTAACCAGC TATAGTGTAC ACTGGGTTCG CCAGCCTCCA GGAAAGGGTC TGGAGTGGCT 120

GGGAGTAATC TGGGCTAGTG GAGGCACAGA TTATAATTCG GCTCTCATGT CCAGACTGAG 180

30 CATCAGCAAA GACAACTCCA AGAGCCAAGT TTTCTTAAAA CTGAACAGTC TGCAAACTGA 240

TGACGCAGCC ATGTACTACT GTGCCAGAGA TCCCCCTTTT TCCTTACTAC GGCTTGACTT 300

CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCA 334

35

(2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 315 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..315
- (D) OTHER INFORMATION: /note= "First base corresponds to  
Kabat position 25"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCCTCTCTGA GTGTGTCAGC AGGAGAGAAG GTCACTATGA GCTGCAAGTC CAGTCAGAGT	60
CTGTTAAACA GTGGAAATCA AAAAAACTAC TTGGCCTGGT ACCAGCAGAA ACCAGGGCAG	120
CCTCCTAAAC TTTTGATCTA CGGGGCATCC ACTAGGGAAT CTGGGGTCCC TGATCGCTTC	180
ACAGGCAGTG GATCTGGAAC CGATTTCACT CTTACCATCA GCAGTGTGCA GGCTGAAGAC	240
CTGGCAGTTT ATTACTGTCA GAATGATCAT AGTTTTCCAT TCACGTTCGG CTCGGGGACA	300
GAGTTGGAAA TAAAA	315

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear



(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10      Ser Tyr Ser Val His  
         1                      5

(2) INFORMATION FOR SEQ ID NO:8:

15      (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20      (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

25

Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met Ser  
1                      5                      10                      15

30      (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- 35      (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr  
1 5 10

10 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu  
25 1 5 10 15

Ala

30 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Ala Ser Thr Arg Glu Ser  
1 5

10 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Asn Val His Ser Phe Pro Phe Thr  
25 1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5           Gln Asn Asp His Ser Phe Pro Phe Thr  
            1                               5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

10           (A) LENGTH: 11 amino acids  
            (B) TYPE: amino acid  
            (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

15           (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

20           Asp Pro Pro Phe Ser Leu Leu Arg Leu Asp Phe  
            1                               5                               10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

25           Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln  
            1                               5                               10                               15

30           Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr  
                                  20                               25                               30

            Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
                                  35                               40                               45

35           Gly Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met  
                                  50                               55                               60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu  
65 70 75 80

Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala  
5 85 90 95

Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

10 Thr Thr Leu Thr Val Ser Ser  
115

(2) INFORMATION FOR SEQ ID NO:16:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 113 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

25 Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly  
1 5 10 15

30 Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
35 40 45

35 Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser  
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
5 85 90 95

Val His Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr Glu Leu Glu Ile  
100 105 110

10 Lys

(2) INFORMATION FOR SEQ ID NO:17:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 60 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGGTGTTGC AGACCCAGGT CTTCATTTCT CTGTTGCTCT GGATCTCTGG TGCCTACGGG 60

30 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 357 base pairs  
(B) TYPE: nucleic acid  
35 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGGTTACCC TCGTGAATC CGGTCCGGCA CTAGTTAAAC CGACCCAGAC CCTGACGTTA 60  
ACCTGCACCG TCTCCGGTTT CTCCCTGACG AGCTATAGTG TACACTGGGT CCGTCAGCCG 120  
10 CCGGGTAAAG GTCTAGAATG GCTGGGTGTA ATATGGGCTA GTGGAGGCAC AGATTATAAT 180  
TCGGCTCTCA TGTCCCGTCT GTCGATATCC AAAGACACCT CCCGTAACCA GGTGTGTTCTG 240  
15 ACCATGACTA ACATGGACCC GGTGACACC GCTACCTACT ACTGCGCTCG AGATCCCCCT 300  
TCTTCCTTAC TACGGCTTGA CTA CTGGGGT CGTGGTACCC CAGTTACCGT GAGCTCA 357

(2) INFORMATION FOR SEQ ID NO:19:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15  
35 Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr  
20 25 30

Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu  
 35 40 45  
 5 Gly Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met  
 50 55 60  
 Ser Arg Leu Ser Ile Ser Lys Asp Thr Ser Arg Asn Gln Val Val Leu  
 65 70 75 80  
 10 Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala  
 85 90 95  
 Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Arg Gly  
 15 100 105 110  
 Thr Pro Val Thr Val Ser Ser  
 115

20 (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 339 base pairs  
 (B) TYPE: nucleic acid  
 25 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATATCGTGA TGACCCAGTC TCCAGACTCG CTAGCTGTGT CTCTGGGCGA GAGGGCCACC 60  
 35 ATCAACTGCA AGAGCTCTCA GAGTCTGTTA AACAGTGGAA ATCAAAAGAA CTA CTGGGCC 120



TGGTATCAGC AGAAACCCGG GCAGCCTCCT AAGTTGCTCA TTTACGGGGC GTCGACTAGG 180  
GAATCTGGGG TACCTGACCG ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC 240  
5 ATCAGCAGCC TGCAGGCTGA AGATGTGGCA GTATACTACT GTCAGAATGT TCATAGTTTT 300  
CCATTCACGT TCGGCGGAGG GACCAAGTTG GAGATCAAA 339

(2) INFORMATION FOR SEQ ID NO:21:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

25

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
20 25 30

30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
50 55 60

35

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Asn  
85 90 95

5 Val His Ser Phe Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
100 105 110

Lys

10 (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

25 GTACATATGC AAGGCTTACA ACCACAATC

29

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGACAGGGCT TACTAGTGGG CCCTCTGGGC TC

32

5 (2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGGTSMARCT KTCTCGAGTC WGG

23

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

25 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

35 CTAACACTCA TTCCTGTTGA AGCTCTTGAC AATGGG

36

(2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCAGATGTGA GCTCGTGATG ACCCAGACTC CA

32

15

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 base pairs

20 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

30 AACCTGCACC GTCTCCGGTT TCTCCCTGAC GAGCTATAGT GTACACTGGG TCCGTCAGCC 60

GCCGGGTAAA GGTCTAGAAT GGCTGGGTGT AATATGGGCT AGTGGAGGCA CAGATTATAA 120

TTCGGCTCTC ATGTCCCGTC 140

35

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATATCGACAG ACGGGACATG AGAGCCGAAT TATAATCTGT GCCTCCACTA GCCCATATTA 60  
CACCCAGCCA TTCTAGACCT TTACCCGGCG GCTGACGGAC CCAGTGTACA CTATAGCTCG 120  
TCAGGGAGAA ACCGGAGACG GTGCAGGTT 149

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGTCGATATC CAAAGACACC TCCCGTAACC AGGTTGTTCT GACCATGACT AACATGGACC 60  
CGGTTGACAC CGCTACCTAC TACTGCGCTC GAGATCCCCC TTCTTCCTTA CTACGGCTTG 120  
ACTACTGGGG TCGTGGTAC 139

## (2) INFORMATION FOR SEQ ID NO:30:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 126 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

15 CACGACCCCA GTAGTCAAGC CGTAGTAAGG AAGAAGGGGG ATCTCGAGCG CAGTAGTAGG 60  
TAGCGGTGTC AACCGGGTCC ATGTTAGTCA TGGTCAGAAC AACCTGGTTA CGGGAGGTGT 120  
20 CTTTGG 126

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 117 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 30 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

35 CTCAGAGTCT GTTAAACAGT GGAAATCAAA AGAACTACTT GGCCTGGTAT CAGCAGAAAC 60

CCGGGCAGCC TCCTAAGTTG CTCATTTACG GGGCGTCGAC TAGGGAATCT GGGGTAC 117

(2) INFORMATION FOR SEQ ID NO:32:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 117 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

- (ii) MOLECULE TYPE: DNA (genomic)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCCAGATTCC CTAGTCGACG CCCC GTAAAT GAGCAACTTA GGAGGCTGCC CGGGTTTCTG 60

CTGATACCAG GCCAAGTAGT TCTTTTGATT TCCACTGTTT AACAGACTCT GAGAGCT 117

20

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 102 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25

- (ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

35 GCTGAAGATG TGGCAGTATA CTA CTGTCAG AATG TTCATA GTTTTCCATT CACGTTCGGC 60

GGAGGGACCA AGTTGGAGAT CAAACGTACT GTGGCGGCGC CA 102

## (2) INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 111 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

15

AGCTTGGCGC CGCCACAGTA CGTTTGATCT CCAACTTGGT CCCTCCGCCG AACGTGAATG 60

GAAAACTATG AACATTCTGA CAGTAGTATA CTGCCACATC TTCAGCCTGC A 111

## 20 (2) INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 82 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGGTGTTGC AGACCCAGGT CTCATTTCT CTGTTGCTCT GGATCTCTGG TGCCTACGGG 60

35

CAGGTTCAAC TGAAAGAGTC AG 82



## (2) INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCCTGACTC TTTCAGTTGA ACCTGCCCCGT AGGCACCAGA GATCCAGAGC AACAGAGAAA 60  
TGAAGACCTG GGTCTGCAAC ACCATGTTG 89

## (2) INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CAAGGCACCA CTCTCACAGT CTCCTCAGCT AGTACGAAGG GCCCA 45

## (2) INFORMATION FOR SEQ ID NO:38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AGCTTGGGCC CTTCGTACTA GCTGAGGAGA CTGTGAGTGG TGC

43

(2) INFORMATION FOR SEQ ID NO:39:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: DNA (genomic)

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ATCGTGATGA CCCAGTCTCC ATCCTCCC

28

30

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TCAGGGAGGA TGGAGACTGG GTCATCACGA T

31

(2) INFORMATION FOR SEQ ID NO:41:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCGGGGGACA GAGTTGGAAA TAAAACGTAC TGTGGCGGCG CCA

43

25 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AGCTTGCGC CGCCACAGTA CGTTTTATTT CCAACTCTGT CC

42

(2) INFORMATION FOR SEQ ID NO:43:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTAGCCACCA CCACCACCAC CACTAA

26

20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

25

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTAGTTAGTG GTGGTGGTGG TGGTGG

26

35

(2) INFORMATION FOR SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 113 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly  
 1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
 20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
 50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
 65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
 85 90 95

Asp His Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile  
 100 105 110

Lys

## (2) INFORMATION FOR SEQ ID NO:46:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly  
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
85 90 95

Asp Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile  
100 105 110

Lys

## (2) INFORMATION FOR SEQ ID NO:47:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gln Asn Asp His Ser Tyr Pro Phe Thr  
1 5

## (2) INFORMATION FOR SEQ ID NO:48:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gln Asn Asp Tyr Ser Tyr Pro Phe Thr  
1 5

## (2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6285 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: circular

5

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

15

GACGTCGCGG	CCGCTCTAGG	CCTCCAAAAA	AGCCTCCTCA	CTACTTCTGG	AATAGCTCAG	60
AGGCCGAGGC	GGCCTCGGCC	TCTGCATAAA	TAAAAAAAT	TAGTCAGCCA	TGCATGGGGC	120
GGAGAATGGG	CGGAACTGGG	CGGAGTTAGG	GGCGGGATGG	GCGGAGTTAG	GGGCGGGACT	180
ATGGTTGCTG	ACTAATTGAG	ATGCATGCTT	TGCATACTTC	TGCCTGCTGG	GGAGCCTGGG	240
GACTTTCCAC	ACCTGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCATACT	TCTGCCTGCT	300
GGGGAGCCTG	GGGACTTTCC	ACACCCTAAC	TGACACACAT	TCCACAGAAT	TAATTCCCGG	360
GGATCGATCC	GTCGACGTAC	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	GTCATTAGTT	420
CATAGCCCAT	ATATGGAGTT	CCGCGTTACA	TAACTTACGG	TAAATGGCCC	GCCTGGCTGA	480
CCGCCCAACG	ACCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	AGTAACGCCA	540
ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTAC	GGTAAACTGC	CCACTTGGCA	600
GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	CGGTAAATGG	660
CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCCTACTTG	GCAGTACATC	720
TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GGCAGTACAT	CAATGGGCGT	780

35



	GGATAGCGGT TTGACTCACG GGGATTTCCTA AGTCTCCACC CCATTGACGT CAATGGGAGT	840
	TTGTTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAACAACTC CGCCCCATTG	900
5	ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG	960
	AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCGAGGAC GCCAGCAACA TGGTGTGCA	1020
10	GACCCAGGTC TTCATTTCTC TGTGCTCTG GATCTCTGGT GCCTACGGGC AGGTTACCCT	1080
	GCGTGAATCC GGTCCGGCAC TAGTTAAACC GACCCAGACC CTGACGTAA CCTGCACCGT	1140
	CTCCGGTTTC TCCCTGACGA GCTATAGTGT ACACTGGGTC CGTCAGCCGC CGGGTAAAGG	1200
15	TCTAGAATGG CTGGGTGTAA TATGGGCTAG TGGAGGCACA GATTATAATT CGGCTCTCAT	1260
	GTCCCGTCTG TCGATATCCA AAGACACCTC CCGTAACCAG GTTGTCTGA CCATGACTAA	1320
20	CATGGACCCG GTTGACACCG CTACCTACTA CTGCGCTCGA GATCCCCCTT CTTCTTACT	1380
	ACGGCTTGAC TACTGGGGTC GTGGTACCCC AGTTACCGTG AGCTCAGCTA GTACCAAGGG	1440
	CTCATCGGTC TTCCCCCTGG CACCCTCCTC CAAGAGCACC TCTGGGGGCA CAGCGGCCCT	1500
25	GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACG GTGTCGTGGA ACTCAGGCGC	1560
	CCTGACCAGC GGCCTGCACA CCTTCCCGGC TGTCCTACAG TCCTCAGGAC TCTACTCCCT	1620
30	CAGCAGCGTG GTGACCGTGC CCTCCAGCAG CTTGGGCACC CAGACCTACA TCTGCAACGT	1680
	GAATCACAAG CCCAGCAACA CCAAGGTGGA CAAGAGAGTT GAGCCCCAAT CTTGTGACAA	1740
	AACTCACACA TGCCACCGT GCCCAGCACC TGAACCTCTG GGGGGACCGT CAGTCTTCCT	1800
35	CTTCCCCCA AAACCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT	1860
	GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT	1920

	GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT	1980
	GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA	2040
5	GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA	2100
	GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG GAGGAGATGA CCAAGAACCA	2160
10	GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA	2220
	GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG	2280
	CTCCTTCTTC CTCTATAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT	2340
15	CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC	2400
	CCTGTCTCCG GGTAAGTGAG TGTAGTCTAG ATCTACGTAT GATCAGCCTC GACTGTGCCT	2460
20	TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCTTGAC CCTGGAAGGT	2520
	GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG CATCGCATTG TCTGAGTAGG	2580
	TGTCATTCTA TTCTGGGGGG TGGGGTGGGG CAGGACAGCA AGGGGGAGGA TTGGGAAGAC	2640
25	AATAGCAGGC ATGCTGGGGA TGCGGTGGGC TCTATGGAAC CAGCTGGGGC TCGACAGCGC	2700
	TGGATCTCCC GATCCCCAGC TTTGCTTCTC AATTTCTTAT TTGCATAATG AGAAAAAAG	2760
30	GAAAATTAAT TTAAACACCA ATTCAGTAGT TGATTGAGCA AATGCGTTGC CAAAAAGGAT	2820
	GCTTTAGAGA CAGTGTTCTC TGCACAGATA AGGACAAACA TTATTCAGAG GGAGTACCCA	2880
	GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATTCTA GGGAGAAATA TGCTTGTCAT	2940
35	CACCGAAGCC TGATTCCGTA GAGCCACACC TTGGTAAGGG CCAATCTGCT CACACAGGAT	3000

	AGAGAGGGCA GGAGCCAGGG CAGAGCATAT AAGGTGAGGT AGGATCAGTT GTCCTCACA	3060
	TTTGCTTCTG ACATAGTTGT GTTGGGAGCT TGGATAGCTT GGACAGCTCA GGGCTGCGAT	3120
5	TTCGCGCCAA ACTTGACGGC AATCCTAGCG TGAAGGCTGG TAGGATTTTA TCCCCGCTGC	3180
	CATCATGGTT CGACCATTGA ACTGCATCGT CGCCGTGTCC CAAAATATGG GGATTGGCAA	3240
	GAACGGAGAC CTACCCTGGC CTCCGCTCAG GAACGAGTTC AAGTACTTCC AAAGAATGAC	3300
10	CACAACCTCT TCAGTGGAAG GTAAACAGAA TCTGGTGATT ATGGGTAGGA AAACCTGGTT	3360
	CTCCATTCTT GAGAAGAATC GACCTTTAAA GGACAGAATT AATATAGTTC TCAGTAGAGA	3420
15	ACTCAAAGAA CCACCACGAG GAGCTCATTT TCTTGCCAAA AGTTTGGATG ATGCCTTAAG	3480
	ACTTATTGAA CAACCGGAAT TGGCAAGTAA AGTAGACATG GTTTGGATAG TCGGAGGCAG	3540
	TTCTGTTTAC CAGGAAGCCA TGAATCAACC AGGCCACCTT AGACTCTTTG TGACAAGGAT	3600
20	CATGCAGGAA TTTGAAAGTG ACACGTTTTT CCCAGAAATT GATTGGGGA AATATAAACT	3660
	TCTCCCAGAA TACCCAGGCG TCCTCTCTGA GGTCCAGGAG GAAAAAGGCA TCAAGTATAA	3720
25	GTTTGAAGTC TACGAGAAGA AAGACTAACA GGAAGATGCT TTCAAGTTCT CTGCTCCCCT	3780
	CCTAAAGCTA TGCATTTTTA TAAGACCATG GGACTTTTGC TGGCTTTAGA TCAGCCTCGA	3840
	CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCCTC CCCCCTGCCT TCCTTGACCC	3900
30	TGGAAGGTGC CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC	3960
	TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG GGGGAGGATT	4020
35	GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC TATGGAACCA GCTGGGGCTC	4080
	GATCGAGTGT ATGACTGCGG CCGCGATCCC GTCGAGAGCT TGGCGTAATC ATGGTCATAG	4140

	CTGTTTCCTG	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	4200
5	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	4260
	TCACTGCCCCG	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	4320
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	4380
10	CTGCGCTCGG	TCGTTCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	4440
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	4500
	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTTC	ATAGGCTCCG	CCCCCCTGAC	4560
15	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	4620
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	4680
20	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	4740
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	4800
	CCCGTTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	4860
25	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	4920
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	4980
30	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	5040
	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	5100
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	5160
35	CAGTGGAACG	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	5220

ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA 5280

ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA 5340

5 TTTCGTTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC 5400

TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT 5460

TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA 5520

10 TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT 5580

AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTCACG CTCGTCGTTT 5640

15 GGTATGGCTT CATTCAGCTC CGGTTCCTAA CGATCAAGGC GAGTTACATG ATCCCCCATG 5700

TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGGCC 5760

GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC 5820

20 GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG 5880

CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA 5940

25 ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACTCTC AAGGATCTTA 6000

CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT 6060

TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG 6120

30 GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA 6180

AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT 6240

35 AAACAAATAG GGGTCCGCG CACATTTCCC CGAAAAGTGC CACCT 6285

(2) INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5703 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: double

(D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: DNA (genomic)

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

15 GACGTCGCGG CCGCTCTAGG CCTCCAAAAA AGCCTCCTCA CTACTTCTGG AATAGCTCAG 60  
AGGCCGAGGC GGCCTCGGCC TCTGCATAAA TAAAAAAAAT TAGTCAGCCA TGCATGGGGC 120  
GGAGAATGGG CGGAACTGGG CGGAGTTAGG GCGGGGATGG GCGGAGTTAG GGGCGGGACT 180  
20 ATGGTTGCTG ACTAATTGAG ATGCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG 240  
GACTTTCCAC ACCTGGTTGC TGA CTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT 300  
GGGGAGCCTG GGGACTTTCC ACACCCTAAC TGACACACAT TCCACAGAAT TAATTCCCGG 360  
25 GGATCGATCC GTCGACGTAC GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT 420  
CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA 480  
30 CCGCCCAACG ACCCCCCGCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA 540  
ATAGGGACTT TCCATTGACG TCAATGGGTG GACTATTTAC GGTAAACTGC CCACTTGGCA 600  
GTACATCAAG TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG 660  
35 CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC 720

	TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT	780
	GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT	840
5	TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACCTC CGCCCCATTG	900
	ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG	960
	AACCGTCAGA TCGCCTGGAG ACGCCATCGA ATTCATTGAT AGGATCCAGC AAGATGGTGT	1020
10	TGCAGACCCA GGTCTTCATT TCTCTGTTGC TCTGGATCTC TGGTGCCTAC GGGGATATCG	1080
	TGATGACCCA GTCTCCAGAC TCGCTAGCTG TGTCTCTGGG CGAGAGGGCC ACCATCAACT	1140
15	GCAAGAGCTC TCAGAGTCTG TTAAACAGTG GAAATCAAAA GAACTACTTG GCCTGGTATC	1200
	AGCAGAAACC CGGGCAGCCT CCTAAGTTGC TCATTTACGG GCGTCGACT AGGGAATCTG	1260
	GGGTACCTGA CCGATTCACT GGCAGCGGGT CTGGGACAGA TTCTACTCTC ACCATCAGCA	1320
20	GCCTGCAGGC TGAAGATGTG GCAGTATACT ACTGTCAGAA TGTTCATAGT TTTCCATTCA	1380
	CGTTCGGCGG AGGGACCAAG TTGGAGATCA AACGTACTGT GCGGCGCCA TCTGTCTTCA	1440
25	TCTTCCCGCC ATCTGATGAG CAGTTGAAAT CTGGAAGTGC CTCTGTTGTG TGCCTGCTGA	1500
	ATAACTTCTA TCCCAGAGAG GCCAAAGTAC AGTGGAAGGT GGATAACGCC CTCCAATCGG	1560
	GTAACCTCCA GGAGAGTGTC ACAGAGCAGG ACAGCAAGGA CAGCACCTAC AGCCTCAGCA	1620
30	GCACCCCTGAC GCTGAGCAAA GCAGACTACG AGAAACACAA AGTCTACGCC TGCGAAGTCA	1680
	CCCATCAGGG CCTGAGCTCG CCCGTCACAA AGAGCTTCAA CAGGGGAGAG TGTTAATTCT	1740
35	AGATCCGTTA TCTACGTATG ATCAGCCTCG ACTGTGCCTT CTAGTTGCCA GCCATCTGTT	1800
	GTTTGCCCCCT CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC TGTCTTTCC	1860

	TAATAAAATG AGGAAATTGC ATCGCATTGT CTGAGTAGGT GTCATTCTAT TCTGGGGGGT	1920
	GGGGTGGGGC AGGACAGCAA GGGGGAGGAT TGGGAAGACA ATAGCAGGCA TGCTGGGGAT	1980
5	GCGGTGGGCT CTATGGAACC AGCTGGGGCT CGACAGCTCG AGCTAGCTTT GCTTCTCAAT	2040
	TTCTTATTTG CATAATGAGA AAAAAAGGAA AATTAATTTT AACACCAATT CAGTAGTTGA	2100
10	TTGAGCAAAT GCGTTGCCAA AAAGGATGCT TTAGAGACAG TGTTCTCTGC ACAGATAAGG	2160
	ACAAACATTA TTCAGAGGGA GTACCCAGAG CTGAGACTCC TAAGCCAGTG AGTGGCACAG	2220
	CATTCTAGGG AGAAATATGC TTGTCATCAC CGAAGCCTGA TTCCGTAGAG CCACACCTTG	2280
15	GTAAGGGCCA ATCTGCTCAC ACAGGATAGA GAGGGCAGGA GCCAGGGCAG AGCATATAAG	2340
	GTGAGGTAGG ATCAGTTGCT CCTCACATTT GCTTCTGACA TAGTTGTGTT GGGAGCTTGG	2400
20	ATCGATCCAC CATGGTTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC GCTTGGGTGG	2460
	AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG CTGCTCTGAT GCCGCCGTGT	2520
	TCCGGCTGTC AGCGCAGGGG CGCCCGGTTT TTTTGTCAA GACCGACCTG TCCGGTGCCC	2580
25	TGAATGAACT GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GCGTTCCTT	2640
	GCGCAGCTGT GCTCGACGTT GTCCTGAAG CGGGAAGGGA CTGGCTGCTA TTGGGCGAAG	2700
30	TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG	2760
	CTGATGCAAT GCGGCGGCTG CATACTGCTT ATCCGGCTAC CTGCCCATTG GACCACCAAG	2820
	CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG	2880
35	ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC	2940



	GCATGCCCCGA CGGCGAGGAT CTCGTCTGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA	3000
	TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC	3060
5	GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCGAATGGG	3120
	CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT	3180
	ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC	3240
10	GACGCCCAAC CTGCCATCAC GAGATTTCTGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG	3300
	CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT	3360
15	GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA	3420
	TAGCATCACA AATTTACAAA ATAAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGTC	3480
	CAAACATCATC AATGTATCTT ATCATGTCTG GATCGCGGCC GCGATCCCGT CGAGAGCTTG	3540
20	GCGTAATCAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC	3600
	AACATACGAG CCGGAAGCAT AAAGTGTAAG GCCTGGGGTG CCTAATGAGT GAGCTAACTC	3660
25	ACATTAATTG CGTTGCGCTC ACTGCCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG	3720
	CATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG CTCTTCCGCT	3780
	TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC GCGAGCGGT ATCAGCTCAC	3840
30	TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA	3900
	GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT	3960
35	AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC	4020
	CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT	4080

GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG 4140  
 CTTTCTCAAT GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG 4200  
 5 GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT 4260  
 CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG 4320  
 10 ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC 4380  
 GGCTACACTA GAAGGACAGT ATTTGGTATC TCGCTCTGCG TGAAGCCAGT TACCTTCGGA 4440  
 AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTTT 4500  
 15 GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT 4560  
 TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA 4620  
 20 TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC 4680  
 TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT 4740  
 ATCTCAGCGA TCTGTCTATT TC GTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA 4800  
 25 ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA 4860  
 CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA 4920  
 30 AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA 4980  
 GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG 5040  
 GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA 5100  
 35 GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT 5160

5220 GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT  
5280 CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA  
5 5340 TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT  
5400 ACCGCGCCAC ATAGCAGAAC TTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA  
5460 AAACCTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC  
10 5520 AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG  
5580 CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC  
15 5640 CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT  
5700 GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA  
5703 CCT

20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

35 ATCCAAAGAC AACTCCCGTA ACCAGGTTGT TCTGACCATG ACTAACATGG ACCCGGTTGA 60  
CACCGCTACC TACTACTGCG C 81

## (2) INFORMATION FOR SEQ ID NO:52:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 85 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

15 TCGAGCGCAG TAGTAGGTAG CGGTGTCAAC CGGGTCCATG TTAGTCATGG TCAGAACAAC 60  
CTGGTTACGG GAGTTGTCTT TGGAT 85

## 20 (2) INFORMATION FOR SEQ ID NO:53:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 73 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

35 AACCTGCACC GTCTCCGGTT TCTCCCTGAC GAGCTATAGT GTACACTGGA TCCGTCAGCC 60  
GCCGGGTAAA GGT 73

## (2) INFORMATION FOR SEQ ID NO:54:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTAGACCTTT ACCCGGCGGC TGACGGATCC AGTGTACACT ATAGCTCGTC AGGGAGAAAC 60  
CGGAGACGGT GCAGGTT 77

## (2) INFORMATION FOR SEQ ID NO:55:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CTAGCTGTGT CAGCTGGCGA GAGGGCCACC ATCAACTGCA AGAGCT 46

## (2) INFORMATION FOR SEQ ID NO:56:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: DNA (genomic)

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CTTGCA GTTG ATGGTGGCCC TCTCGCCAGC TGACACAG

38

(2) INFORMATION FOR SEQ ID NO:57:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: DNA (genomic)

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TTCGAGGACG CCAGCAACAT GGTGTTGCAG ACCCAGGTCT TCATTTCTCT GTTGCTCTGG

60

30

ATCTCTGGTG CCTACGGGCA GGTCCAACCTG CAGGAGAGCG GTCCAGGTCT TGTGAGACCT

120

AGCCAGACCC TGAGCCTGAC

140

(2) INFORMATION FOR SEQ ID NO:58:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 138 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

10

GTGCCTCCAC TAGCCCATAT TACTCCAAGC CACTCTAGAC CTCGTCCAGG TGGCTGTCTC 60

ACCCAGTGTA CACTATAGCT GGTGAGGGAG AAGCCCGAGA CGGTGCAGGT CAGGCTCAGG 120

15 GTCTGGCTAG GTCTCACA 138

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 143 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

30

GGCTTGGAGT AATATGGGCT AGTGAGGCA CAGATTATAA TTCGGCTCTC ATGTCCAGAC 60

TGAGTATACT GAAAGACAAC AGCAAGAACC AGGTCAGCCT GAGACTCAGC AGCGTGACAG 120

35 CCGCCGACAC CGCGGTCTAT TTC 143

(2) INFORMATION FOR SEQ ID NO:60:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 136 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCAGTGCCAA GCTTGGGCCC TTGGTGGAGG CGCTCGAGAC GGTGACCGTG GTACCTTGTC 60

15

CCCAGTAGTC AAGCCGTAGT AAGGAAGAAG GGGGATCTCG AGCACAGAAA TAGACCGCGG 120

TGTCGGCGGC TGTCAC 136

## 20 (2) INFORMATION FOR SEQ ID NO:61:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CAGGTCCAAC TGCAGGAGAG CGGTCCAGGT CTTGTGAGAC CTAGCCAGAC CCTGAGCCTG 60

35

ACCTGCACCG TCTCGGGCTT CTCCCTCACC AGCTATAGTG TACTTGGGT GAGACAGCCA 120



CCTGGACGAG GTCTAGAGTG GCTTGGAGTA ATATGGGCTA GTGGAGGCAC AGATTATAAT 180  
 TCGGCTCTCA TGTCCAGACT GAGTATACTG AAAGACAACA GCAAGAACCA GGTCAGCCTG 240  
 5 AGACTCAGCA GCGTGACAGC CGCCGACACC GCGGTCTATT ACTGTGCTCG GGATCCCCCT 300  
 TCTTCCTTAC TACGGCTTGA CTACTGGGGA CAAGGTACCA CGGTCACCGT CTCGAGC 357

(2) INFORMATION FOR SEQ ID NO:62:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
 1 5 10 15

25

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr  
 20 25 30

30

Ser Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Leu  
 35 40 45

Gly Val Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn Ser Ala Leu Met  
 50 55 60

35

Ser Arg Leu Ser Ile Leu Lys Asp Asn Ser Lys Asn Gln Val Ser Leu  
 65 70 75 80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

5 Arg Asp Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

Thr Thr Val Thr Val Ser Ser  
115

10 (2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

AGGACGCCAG CAACATGGTG TTGCAGAC

28

25

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TGCCAAGCTT GGGCCCTTGG TGGAGGCGCT CGAGAC

36

5 (2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GACCATGATT ACGAATTCGT AGTCGGATAT CGTGATGACC CAGAGCCCAA GCAGCCTGAG 60

20

CGCTAGCGTG GGTGACAGAG TGACCATCAC CTGTAAGAGC TCTCAGAGTC TGTAAACAG 120

T 121

25 (2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGATTCCCTA GTCGATGCCC CGTAGATCAG CAGCTTTGGA GCCTTACCGG GTTTCTGCTG 60

ATACCAGGCC AAGTAGTTCT TTTGATTTC ACTGTTTAAC AGACTCTGAG AGCTCT 116

5

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 base pairs

10

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

20 TCTACGGGGC ATCGACTAGG GAATCTGGGG TACCAGATAG ATTCAGCGGT AGCGGTAGCG 60

GAACCGACTT CACCTTCACC ATCAGCAGCC TGCAGCCAGA GGACATCGCC ACCTAC 116

(2) INFORMATION FOR SEQ ID NO:68:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TCGATGCCAA GCTTGGCGCC GCCACAGTAC GTTTGATCTC CACCTTGGTC CCTTGTCCGA 60  
ACGTGAATGG AAAACTATGA ACATTCTGGC AGTAGTAGGT GCGGATGTCC TCTGGCT 117

5 (2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs  
(B) TYPE: nucleic acid  
10 (C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GATATCGTGA TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC 60  
20 ATCACCTGTA AGAGCTCTCA GAGTCTGTTA AACAGTGGAA ATCAAAAGAA CTACTTGGCC 120  
TGGTATCAGC AGAAACCCGG TAAGGCTCCA AAGCTGCTGA TCTACGGGGC ATCGACTAGG 180  
25 GAATCTGGGG TACCAGATAG ATTCAGCGGT AGCGGTAGCG GAACCGACTT CACCTTCACC 240  
ATCAGCAGCC TGCAGCCAGA GGACATCGCC ACCTACTACT GCCAGAATGT TCATAGTTTT 300  
CCATTACGT TCGGACAAGG GACCAAGGTG GAGATCAAA 339

30

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 113 amino acids  
35 (B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

10

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
20 25 30

15

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys  
35 40 45

Ala Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
50 55 60

20

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr  
65 70 75 80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Asn  
85 90 95

25

Val His Ser Phe Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile  
100 105 110

Lys

30

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GATTACGAAT TCGTAGTCGG ATAT

24

10

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

25 TGCCAAGCTT GCGCCGCCA CAGT

24

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CTAGTGCGGG TGACCGAGTG ACCATCACCT GTAAGAGCT

39

5

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

10

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTACAGGTG ATGGTCACTC GGTCACCCGC A

31

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 66 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GGTCTATTAC TGTGCTCGGG ATCCCCCTTC TTCCTTACTA CGGCTTGACT ACTGGGGACA

60

AGGTAC

66



## (2) INFORMATION FOR SEQ ID NO:76:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 64 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

15 CTTGTCCCCA GTAGTCAAGC CGTAGTAAGG AAGAAGGGGG ATCCCGAGCA CAGTAATAGA 60  
CCGC 64

## WHAT IS CLAIMED IS:

1. A rodent neutralizing monoclonal antibody specific for human interleukin-5 and having a binding affinity characterized by a dissociation constant equal to or less than about  $3.5 \times 10^{-11}$  M.
2. The monoclonal antibody according to claim 1 which is a murine monoclonal antibody.
3. The monoclonal antibody according to claim 1 which is a rat monoclonal antibody.
4. The monoclonal antibody according to claim 2 which comprises the light chain amino acid sequence of SEQ ID NO: 16, and the heavy chain amino acid sequence of SEQ ID NO: 15.
5. The monoclonal antibody according to claim 1 having the identifying characteristics of 2B6, 2E3, 2F2 or 4A6.
6. A hybridoma having the identifying characteristics of cell line SK119-2B6.206.75(1), SK119-2E3.39.40.2, SK119-2F2.37.80.12 or 4A6(1)G1F7.
7. A neutralizing Fab fragment or  $F(ab')_2$  fragment thereof, produced by deleting the Fc region of the monoclonal antibody of claim 1.
8. A neutralizing Fab fragment or  $F(ab')_2$  fragment thereof, produced by chain shuffling whereby the Fd heavy chain of the monoclonal antibody of claim 1 is expressed in a murine light chain filamentous phage Fab display library.
9. A neutralizing Fab fragment or  $F(ab')_2$  fragment thereof, produced by chain shuffling whereby the light chain of the monoclonal antibody of claim 1 is expressed in a murine heavy chain filamentous phage Fab display library.
10. An altered antibody comprising a heavy chain and a light chain, wherein the framework regions of said heavy and light chains are derived from at least one selected antibody and the amino acid sequences of the complementarity

determining regions of each said chain are derived from the monoclonal antibody of claim 1.

11. The altered antibody according to claim 10 wherein said amino acid  
5 sequences of the complementarity determining regions of the heavy chain are:

- (a) SEQ ID NO: 7, 8, 9; or
- (b) SEQ ID NO: 7, 8 14.

12. The altered antibody according to claim 10 wherein said amino acid  
10 sequences of the complementarity determining regions of the light chain are:

- (a) SEQ ID NO: 10, 11, 12; or
- (b) SEQ ID NO: 10, 11, 13.

13. The altered antibody according to claim 10 wherein said framework  
15 regions of said heavy chain comprise: amino acids 1-30, 36-49, 66-97 and 109-119 of SEQ ID NO: 19.

14. The altered antibody according to claim 10 wherein said framework  
20 regions of said light chain comprise: amino acids 1-23, 41-55, 63-94 and 104-113 of SEQ ID NO: 21.

15. An immunoglobulin heavy chain complementarity determining  
region (CDR), the amino acid sequence of which is selected from the group  
consisting of :

- 25
- (a) SEQ ID NO: 7,
  - (b) SEQ ID NO: 8,
  - (c) SEQ ID NO: 9, and
  - (d) SEQ ID NO: 14.

30 16. An immunoglobulin light chain complementarity determining region (CDR), the amino acid sequence of which is selected from the group consisting of :

- 35
- (a) SEQ ID NO: 10,
  - (b) SEQ ID NO: 11,
  - (c) SEQ ID NO: 12,
  - (d) SEQ ID NO: 13,
  - (e) SEQ ID NO: 47, and
  - (f) SEQ ID NO: 48.

17. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 15.

5 18. A nucleic acid molecule encoding the immunoglobulin complementarity determining region (CDR) of claim 16.

10 19. A chimeric antibody comprising a heavy chain and a light chain, said antibody characterized by a dissociation constant equal or less than about  $3.5 \times 10^{-11}$  M for human interleukin-5, wherein the constant regions of said heavy and light chains are derived from at least one selected antibody and the amino acid sequences of the variable regions of each said chain are derived from the monoclonal antibody of claim 1.

15 20. The antibody according to claim 19 wherein the constant regions are selected from human immunoglobulins.

20 21. A pharmaceutical composition comprising the altered antibody of claim 10 and a pharmaceutically acceptable carrier.

22. A method of treating conditions associated with excess eosinophil production in a human comprising the step of administering to said human in need thereof an effective amount of the altered antibody of claim 10.

25 23. The method of claim 22 wherein said condition associated with excess eosinophil production is asthma.

30 24. The method of claim 22 wherein said condition associated with excess eosinophil production is allergic rhinitis.

25. The method of claim 22 wherein said condition associated with excess eosinophil production is atopic dermatitis.

35 26. An isolated nucleic acid sequence which is selected from the group consisting of:

- (a) a nucleic acid sequence encoding the altered antibody of claim 10;
- (b) a nucleic acid sequence complementary to (a); and

(c) a fragment or analog of (a) or (b) which encodes a protein characterized by having a specificity for human interleukin-5; wherein said sequence optionally contains a restriction site.

5           27. The isolated nucleic acid sequence according to claim 26, wherein the sequence encoding the humanized heavy chain variable region which comprises the nucleic acid sequence of SEQ ID NO: 18.

10           28. The isolated nucleic acid sequence according to claim 26, wherein the sequence encoding the humanized heavy chain variable region which comprises the nucleic acid sequence of SEQ ID NO: 61.

15           29. The isolated nucleic acid sequence according to claim 26, wherein the sequence encoding the humanized light chain variable region which comprises the nucleic acid sequence of SEQ ID NO: 20.

20           30. The isolated nucleic acid sequence according to claim 26, wherein the sequence encoding the humanized light chain variable region which comprises the nucleic acid sequence of SEQ ID NO: 69.

20           31. A recombinant plasmid comprising the nucleic acid sequence of claim 26.

25           32. A host cell transfected with the recombinant plasmid of claim 31.

30           33. A process for producing an altered antibody specific for human interleukin-5 comprising culturing a cell line transfected with the recombinant plasmid of claim 31 under the control of selected regulatory sequences capable of directing the expression thereof in said cells.

35           34. A method to assess the presence or absence of human IL-5 in a human which comprises obtaining a sample of biological fluid from a patient and allowing the monoclonal antibody of claim 1 to come in contact with such sample under conditions such that an IL-5/monoclonal antibody complex can form and detecting the presence or absence of said IL-5/monoclonal antibody complex.

35. A method for aiding in the diagnosis of allergies and other conditions associated with excess eosinophil production comprising the steps of determining the amount of human IL-5 in a sample of a patient according to the method of claim 34 and comparing that to the mean amount of human IL-5 in the normal population, 5 whereby the presence of significantly elevated amount of human IL-5 in the patient is an indication of allergies and other conditions associated with excess eosinophil production.

36. A hybridoma having the identifying characteristics of cell line 10 SK119-24G9.8.20.5 or 5D3(1)F5D6.

37. A monoclonal antibody produced by the hybridoma of claim 36.

FIGURE 1

## 2B6 Heavy Chain Variable Region DNA Sequence.

1		CCT GGC	30
	Gln Val Gln Leu Lys Glu Ser Gly Pro Gly		
31	CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC	60	
	Leu Val Ala Pro Ser Gln Ser Leu Ser Ile		
61	ACT TGC ACT GTC TCT GGG TTT TCA TTA ACC	90	
	Thr Cys Thr Val Ser Gly Phe Ser Leu Thr		
91	AGC TAT AGT GTA CAC TGG GTT CGC CAG CCT	120	
	Ser Tyr Ser Val His Trp Val Arg Gln Pro		
121	CCA GGA AAG GGT CTG GAG TGG CTG GGA GTA	150	
	Pro Gly Lys Gly Leu Glu Trp Leu Gly Val		
151	ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT	180	
	Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn		
181	TCG GCT CTC ATG TCC AGA CTG AGC ATC AGC	210	
	Ser Ala Leu Met Ser Arg Leu Ser Ile Ser		
211	AAA GAC AAC TCC AAG AGC CAA GTT TTC TTA	240	
	Lys Asp Asn Ser Lys Ser Gln Val Phe Leu		
241	AAA CTG AAC AGT CTG CAA ACT GAT GAC ACA	270	
	Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr		
271	GCC ATG TAC TAC TGT GCC AGA GAT CCC CCT	300	
	Ala Met Tyr Tyr Cys Ala Arg Asp Pro Pro		
301	TCT TCC TTA CTA CGG CTT GAC TAC TGG GGC	330	
	Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly		
331	CAA GGC ACC ACT CTC ACA GTC TCC TCA	357	
	Gln Gly Thr Thr Leu Thr Val Ser Ser		

FIGURE 2

## 2B6 Light Chain Variable Region DNA Sequence

1		TCC TCC	30
	Asp Ile Val Met Thr Gln Ser Pro Ser Ser		
31	CTG AGT GTG TCA GCA GGA GAG AAG GTC ACT	60	
	Leu Ser Val Ser Ala Gly Glu Lys Val Thr		
61	ATG AGC TGC AAG TCC AGT CAG AGT CTG TTA	90	
	Met Ser Cys Lys Ser Ser Gln Ser Leu Leu		
91	AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC	120	
	Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala		
121	TGG TAC CAG CAG AAA CCA GGG CAG CCT CCT	150	
	Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro		
151	AAA CTT TTG ATC TAC GGG GCA TCC ACT AGG	180	
	Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg		
181	GAA TCT GGG GTC CCT GAT CGC TTC ACA GGC	210	
	Glu Ser Gly Val Pro Asp Arg Phe Thr Gly		
211	AGT GGA TCT GGA ACC GAT TTC ACT CTT TCC	240	
	Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser		
241	ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA	270	
	Ile Ser Ser Val Gln Ala Glu Asp Leu Ala		
271	GTT TAT TAC TGT CAG AAT GTT CAT AGT TTT	300	
	Val Tyr Tyr Cys Gln Asn Val His Ser Phe		
301	CCA TTC ACG TTC GGC TCG GGG ACA GAG TTG	330	
	Pro Phe Thr Phe Gly Ser Gly Thr Glu Leu		
331	GAA ATA AAA	339	
	Glu Ile Lys		



FIGURE 3  
2F2 Heavy Chain Variable Region DNA Sequence

1	CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG	30
	Pro Gly Leu Val Ala Pro Ser Gln Ser Leu	
31	TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA	60
	Ser Ile Thr Cys Thr Val Ser Gly Phe Ser	
61	TTA ACC AGT TAT AGT GTA CAC TGG GTT CGC	90
	Leu Thr Ser Tyr Ser Val His Trp Val Arg	
91	CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG	120
	Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu	
121	GGA GTA ATA TGG GCT AGT GGA GGC ACA GAT	150
	Gly Val Ile Trp Ala Ser Gly Gly Thr Asp	
151	TAT AAT TCG GCT CTC ATG TCC AGA CTG AGC	180
	Tyr Asn Ser Ala Leu Met Ser Arg Leu Ser	
181	ATC AGC AAA GAC AAC TCC AAG AGC CAA GTT	210
	Ile Ser Lys Asp Asn Ser Lys Ser Gln Val	
211	TTC TTA AAA CTG AAC AGT CTG CGA ACT GAT	240
	Phe Leu Lys Leu Asn Ser Leu Arg Thr Asp	
241	GAC ACA GCC ATG TAC TAC TGT GCC AGA GAT	270
	Asp Thr Ala Met Tyr Tyr Cys Ala Arg Asp	
271	CCC CCT TCT TCC TTA CTA CGG CTT GAC TAC	300
	Pro Pro Ser Ser Leu Leu Arg Leu Asp Tyr	
301	TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC	330
	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser	
331	TCA	333
	Ser	

FIGURE 4

## 2F2 Light Chain Variable Region DNA Sequence

1	TCC TCC CTC AGT GTG TCH GCH GGA GAG AAG	30
	Ser Ser Leu Ser Val Ser Ala Gly Glu Lys	
31	GTC ACT ATG AGC TGC AAG TCC AGT CAG AGT	60
	Val Thr Met Ser Cys Lys Ser Ser Gln Ser	
61	CTA TTA AAC AGT GGA AAT CAA AAG AAC TAC	90
	Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr	
91	TTG GCC TGG TAC CAA CAG AAA CCA GGG CAG	120
	Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln	
121	CCT CCT AAA CTT TTG ATC TAC GGG GCA TCC	150
	Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser	
151	ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC	180
	Thr Arg Glu Ser Gly Val Pro Asp Arg Phe	
181	ACA GGC AGT GGA TCT GGA ACC GAT TTC ACT	210
	Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr	
211	CTT ACC ATC AGC AGT GTG CAG GCT GAA GAC	240
	Leu Thr Ile Ser Ser Val Gln Ala Glu Asp	
241	CTG GCA GTT TAT TAC TGT CAG AAT GAT CAT	270
	Leu Ala Val Tyr Tyr Cys Gln Asn Asp His	
271	AGT TTT CCA TTC ACG TTC GGC TCG GGG ACA	300
	Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr	
301	GAG TTG GAA ATA AAA	315
	Glu Leu Glu Ile Lys	

FIGURE 5

## 2E3 Heavy Chain Variable Region DNA Sequence

1	CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG	30
	Pro Gly Leu Val Ala Pro Ser Gln Ser Leu	
31	TCC ATC ACT TGC ACT GTC TCT GGG TTT TCA	60
	Ser Ile Thr Cys Thr Val Ser Gly Phe Ser	
61	TTA ACC AGC TAT AGT GTA CAC TGG GTT CGC	90
	Leu Thr Ser Tyr Ser Val His Trp Val Arg	
91	CAG CCT CCA GGA AAG GGT CTG GAG TGG CTG	120
	Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu	
121	GGA GTA ATC TGG GCT AGT GGA GGC ACA GAT	150
	Gly Val Ile Trp Ala Ser Gly Gly Thr Asp	
151	TAT AAT TCG GCT CTC ATG TCC AGA CTG AGC	180
	Tyr Asn Ser Ala Leu Met Ser Arg Leu Ser	
181	ATC AGC AAA GAC AAC TCC AAG AGC CAA GTT	210
	Ile Ser Lys Asp Asn Ser Lys Ser Gln Val	
211	TTC TTA AAA CTG AAC AGT CTG CAA ACT GAT	240
	Phe Leu Lys Leu Asn Ser Leu Gln Thr Asp	
241	GAC GCA GCC ATG TAC TAC TGT GCC AGA GAT	270
	Asp Ala Ala Met Tyr Tyr Cys Ala Arg Asp	
271	CCC CCT TTT TCC TTA CTA CGG CTT GAC TTC	300
	Pro Pro Phe Ser Leu Leu Arg Leu Asp Phe	
301	TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC	330
	Trp Gly Gln Gly Thr Thr Leu Thr Val Ser	
331	TCA	333
	Ser	

FIGURE 6

## 2E3 Light Chain Variable Region DNA Sequence

1	TCC TCT CTG AGT GTG TCA GCA GGA GAG AAG	30
	Ser Ser Leu Ser Val Ser Ala Gly Glu Lys	
31	GTC ACT ATG AGC TGC AAG TCC AGT CAG AGT	60
	Val Thr Met Ser Cys Lys Ser Ser Gln Ser	
61	CTG TTA AAC AGT GGA AAT CAA AAA AAC TAC	90
	Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr	
91	TTG GCC TGG TAC CAG CAG AAA CCA GGG CAG	120
	Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln	
121	CCT CCT AAA CTT TTG ATC TAC GGG GCA TCC	150
	Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser	
151	ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC	180
	Thr Arg Glu Ser Gly Val Pro Asp Arg Phe	
181	ACA GGC AGT GGA TCT GGA ACC GAT TTC ACT	210
	Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr	
211	CTT ACC ATC AGC AGT GTG CAG GCT GAA GAC	240
	Leu Thr Ile Ser Ser Val Gln Ala Glu Asp	
241	CTG GCA GTT TAT TAC TGT CAG AAT GAT CAT	270
	Leu Ala Val Tyr Tyr Cys Gln Asn Asp His	
271	AGT TTT CCA TTC ACG TTC GGC TCG GGG ACA	300
	Ser Phe Pro Phe Thr Phe Gly Ser Gly Thr	
301	GAG TTG GAA ATA AAA	315
	Glu Leu Glu Ile Lys	

FIGURE 7

## 2B6 CDRs

Heavy Chain 1: SYSVH  
Heavy Chain 2: VIWASGGTDYNSALMS  
Heavy Chain 3: DPPSLLRLDY

Light Chain 1: KSSQSLLNSGNQKNYLA  
Light Chain 2: GASTRES  
Light Chain 3: QNVHSFPFT

## 2F2 CDRs

Heavy Chain 1: SYSVH  
Heavy Chain 2: VIWASGGTDYNSALMS  
Heavy Chain 3: DPPSLLRLDY

Light Chain 1: KSSQSLLNSGNQKNYLA  
Light Chain 2: GASTRES  
Light Chain 3: QNDHSFPFT

## 2E3 CDRs

Heavy Chain 1: SYSVH  
Heavy Chain 2: VIWASGGTDYNSALMS  
Heavy Chain 3: DPPFSLLRLDF

Light Chain 1: KSSQSLLNSGNQKNYLA  
Light Chain 2: GASTRES  
Light Chain 3: QNDHSFPFT

FIGURE 8

IL5 Humanized Heavy Chain Variable Region:

1	CAG GTT ACC CTG CGT GAA TCC GGT CCG GCA	30
	Gln Val Thr Leu Arg Glu Ser Gly Pro Ala	
31	CTA GTT AAA CCG ACC CAG ACC CTG ACG TTA	60
	Leu Val Lys Pro Thr Gln Thr Leu Thr Leu	
61	ACC TGC ACC TTC TCC GGT TTC TCC CTG ACG	90
	Thr Cys Thr Phe Ser Gly Phe Ser Leu Thr	
91	AGC TAT AGT GTA CAC TGG ATC CGT CAG CCG	120
	Ser Tyr Ser Val His Trp Ile Arg Gln Pro	
121	CCG GGT AAA GGT CTG GAG TGG CTG GGT GTA	150
	Pro Gly Lys Gly Leu Glu Trp Leu Gly Val	
151	ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT	180
	Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn	
181	TCG GCT CTC ATG TCC CGT CTG ACG ATA TCC	210
	Ser Ala Leu Met Ser Arg Leu Thr Ile Ser	
211	AAA GAC ACC TCC CGT AAC CAG GTT GTT CTG	240
	Lys Asp Thr Ser Arg Asn Gln Val Val Leu	
241	ACC ATG ACT AAC ATG GAC CCG GTT GAC ACC	270
	Thr Met Thr Asn Met Asp Pro Val Asp Thr	
271	GCT ACC TAC TAC TGC GCT CGA GAT CCC CCT	300
	Ala Thr Tyr Tyr Cys Ala Arg Asp Pro Pro	
301	TCT TCC TTA CTA CGG CTT GAC TAC TGG GGT	330
	Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly	
331	CGT GGT ACC CCA GTT ACC GTG AGC TCA	357
	Arg Gly Thr Pro Val Thr Val Ser Ser	

FIGURE 9

## Humanized Light Chain Variable Region

1	GAT ATC GTG ATG ACC CAG TCT CCA GAC TCG	30
	Asp Ile Val Met Thr Gln Ser Pro Asp Ser	
31	CTA GCT GTG TCT CTG GGC GAG AGG GCC ACC	60
	Leu Ala Val Ser Leu Gly Glu Arg Ala Thr	
61	ATC AAC TGC AAG AGC TCT CAG AGT CTG TTA	90
	Ile Asn Cys Lys Ser Ser Gln Ser Leu Leu	
91	AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC	120
	Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala	
121	TGG TAT CAG CAG AAA CCC GGG CAG CCT CCT	150
	Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro	
151	AAG TTG CTC ATT TAC GGG GCG TCG ACT AGG	180
	Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg	
181	GAA TCT GGG GTA CCT GAC CGA TTC AGT GGC	210
	Glu Ser Gly Val Pro Asp Arg Phe Ser Gly	
211	AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC	240
	Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr	
241	ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA	270
	Ile Ser Ser Leu Gln Ala Glu Asp Val Ala	
271	GTA TAC TAC TGT CAG AAT GTT CAT AGT TTT	300
	Val Tyr Tyr Cys Gln Asn Val His Ser Phe	
301	CCA TTC ACG TTC GGC GGA GGG ACC AAG TTG	330
	Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu	
331	GAG ATC AAA	339
	Glu Ile Lys	

FIGURE 10

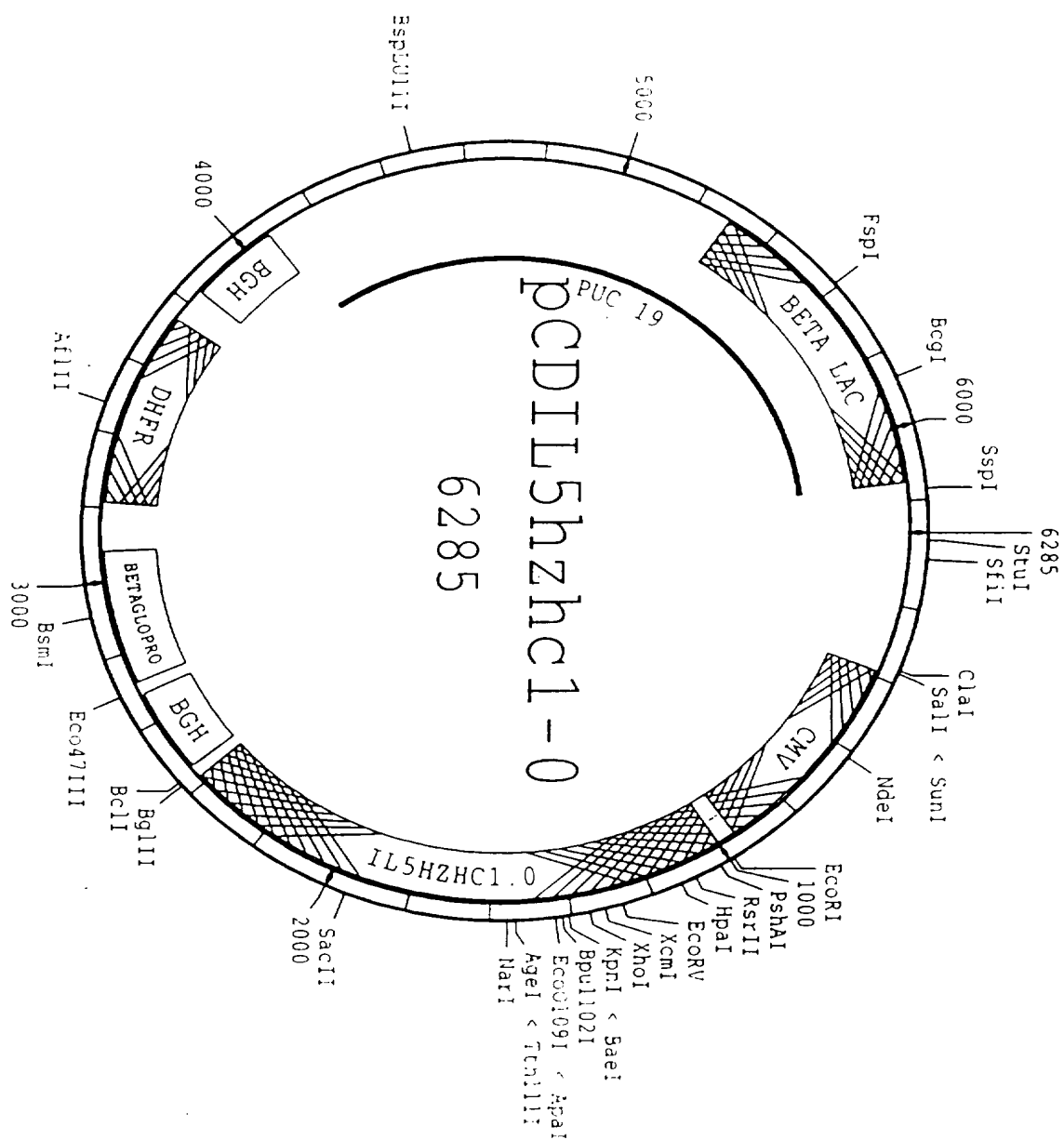




FIGURE 11

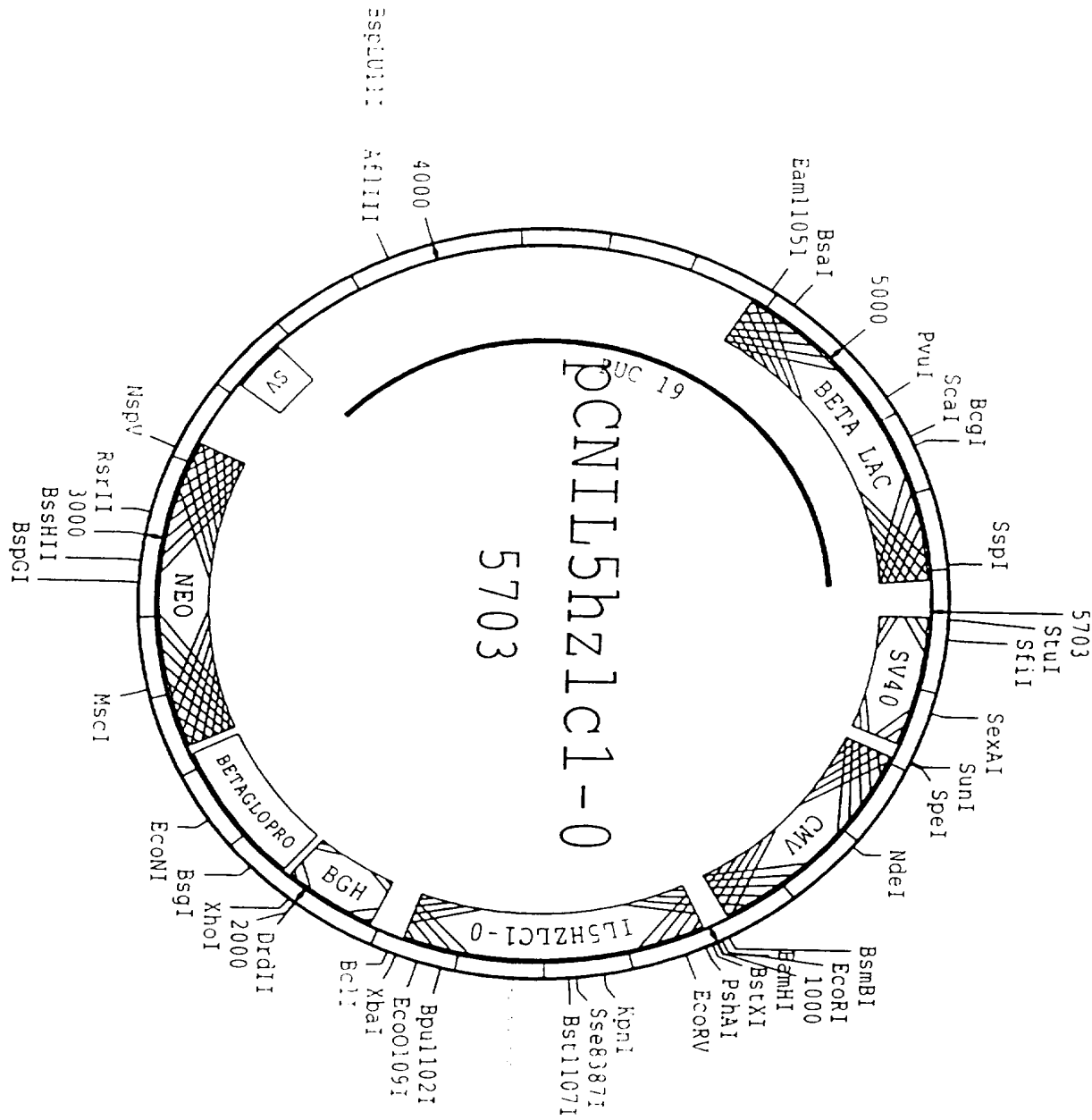


FIGURE 12

## IL5 NEM Humanized Heavy Chain Variable Region

1	CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT	30
	Gln Val Gln Leu Gln Glu Ser Gly Pro Gly	
31	CTT GTG AGA CCT AGC CAG ACC CTG AGC CTG	60
	Leu Val Arg Pro Ser Gln Thr Leu Ser Leu	
61	ACC TGC ACC GTC TCG GGC TTC TCC CTC ACC	90
	Thr Cys Thr Val Ser Gly Phe Ser Leu Thr	
91	AGC TAT AGT GTA CAC TGG GTG AGA CAG CCA	120
	Ser Tyr Ser Val His Trp Val Arg Gln Pro	
121	CCT GGA CGA GGT CTA GAG TGG CTT GGA GTA	150
	Pro Gly Arg Gly Leu Glu Trp Leu Gly Val	
151	ATA TGG GCT AGT GGA GGC ACA GAT TAT AAT	180
	Ile Trp Ala Ser Gly Gly Thr Asp Tyr Asn	
181	TCG GCT CTC ATG TCC AGA CTG TCA ATA CTG	210
	Ser Ala Leu Met Ser Arg Leu Ser Ile Leu	
211	AAA GAC AAC AGC AAG AAC CAG GTC AGC CTG	240
	Lys Asp Asn Ser Lys Asn Gln Val Ser Leu	
241	AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC	270
	Arg Leu Ser Ser Val Thr Ala Ala Asp Thr	
271	GCG GTC TAT TAC TGT GCT CGG GAT CCC CCT	300
	Ala Val Tyr Tyr Cys Ala Arg Asp Pro Pro	
301	TCT TCC TTA CTA CGG CTT GAC TAC TGG GGA	330
	Ser Ser Leu Leu Arg Leu Asp Tyr Trp Gly	
331	CAA GGT ACC ACG GTC ACC GTC TCG AGC	357
	Gln Gly Thr Thr Val Thr Val Ser Ser	

FIGURE 13

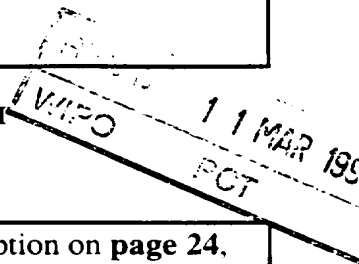
## IL5 REI Humanized Light Chain Variable Region

1	GAT ATC GTG ATG ACC CAG AGC CCA AGC AGC	30
	Asp Ile Val Met Thr Gln Ser Pro Ser Ser	
31	CTG AGC GCT AGC GTG GGT GAC AGA GTG ACC	60
	Leu Ser Ala Ser Val Gly Asp Arg Val Thr	
61	ATC ACC TGT AAG AGC TCT CAG AGT CTG TTA	90
	Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu	
91	AAC AGT GGA AAT CAA AAG AAC TAC TTG GCC	120
	Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala	
121	TGG TAT CAG CAG AAA CCC GGT AAG GCT CCA	150
	Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro	
151	AAG CTG CTG ATC TAC GGG GCA TCG ACT AGG	180
	Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg	
181	GAA TCT GGG GTA CCA GAT AGA TTC AGC GGT	210
	Glu Ser Gly Val Pro Asp Arg Phe Ser Gly	
211	AGC GGT AGC GGA ACC GAC TTC ACC TTC ACC	240
	Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr	
241	ATC AGC AGC CTG CAG CCA GAG GAC ATC GCC	270
	Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala	
271	ACC TAC TAC TGC CAG AAT GTT CAT AGT TTT	300
	Thr Tyr Tyr Cys Gln Asn Val His Ser Phe	
301	CCA TTC ACG TTC GGA CAA GGG ACC AAG GTG	330
	Pro Phe Thr Phe Gly Gln Gly Thr Lys Val	
331	GAG ATC AAA	339
	Glu Ile Lys	

Applicant's or agent's file reference number <b>P50282-2</b>	International Application No.
---	-------------------------------

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

(PCT Rule 13bis)



**A.** The indications made below relate to the microorganism referred to in the description on **page 24, lines 21-25**

**B. IDENTIFICATION OF DEPOSIT** Further deposits are identified on an additional sheet ☐

Name of depositary institution

**American Type Culture Collection (ATCC)**

Address of depositary institution (including postal code and country)

**12301 Parklawn Drive**

**Rockville, MD**

**USA 20852**

Date of Deposit

**21 December 1994**

Accession Number

**HB 11783**

**C. ADDITIONAL INDICATIONS** (leave blank if not applicable) This information is continued on an additional sheet ☐

In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)

**All**

**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Applicant's or agent's file reference number <b>P50282-2</b>	International Application No. <b>P50282-2</b>
---	--

# **INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

**(PCT Rule 13bis)**

A. The indications made below relate to the microorganism referred to in the description on <b>page 24, lines 26-30</b>
---

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet <input type="checkbox"/>
Name of depositary institution <b>American Type Culture Collection (ATCC)</b>	
Address of depositary institution (including postal code and country) <b>12301 Parklawn Drive Rockville, MD USA 20852</b>	

Date of Deposit <b>21 December 1994</b>	Accession Number <b>HB 11782</b>
--	-------------------------------------

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.	

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)
<b>All</b>

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Applicant's or agent's file reference number <b>P50282-2</b>	International Application No. <b>75/000082</b>
---	--

# **INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on <b>page 24, lines 31-35</b>
---

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet <input type="checkbox"/>
Name of depositary institution <b>American Type Culture Collection (ATCC)</b>	
Address of depositary institution (including postal code and country) <b>12301 Parklawn Drive Rockville, MD USA 20852</b>	

Date of Deposit <b>21 December 1994</b>	Accession Number <b>HB 11781</b>
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C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.	

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)
<b>All</b>

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Applicant's or agent's file reference number <b>P50282-2</b>	International Application No. <b>102</b>
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# **INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on **page 24, lines 36 through page 25, line 3**

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution  
**American Type Culture Collection (ATCC)**

Address of depositary institution (including postal code and country)  
**12301 Parklawn Drive  
Rockville, MD  
USA 20852**

Date of Deposit <b>21 December 1994</b>	Accession Number <b>HB 11780</b>
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C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐

In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

**All**

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

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Authorized officer		Authorized officer	

Applicant's or agent's file reference number <b>P50282-2</b>	International Application No. <b>1500000</b>
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# **INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

**(PCT Rule 13bis)**

A. The indications made below relate to the microorganism referred to in the description on <b>page 25, lines 9-13</b>
--

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet <input type="checkbox"/>
Name of depositary institution <b>American Type Culture Collection (ATCC)</b>	
Address of depositary institution (including postal code and country) <b>12301 Parklawn Drive Rockville, MD USA 20852</b>	

Date of Deposit <b>08 June 1995</b>	Accession Number <b>HB 11942</b>
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C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.	

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)
<b>All</b>

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")

<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer



Applicant's or agent's file reference number <b>P50282-2</b>	International Application No.
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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on <b>page 25, lines 4-8</b>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <b>American Type Culture Collection (ATCC)</b>	
Address of depositary institution (including postal code and country) <b>12301 Parklawn Drive Rockville, MD USA 20852</b>	
Date of Deposit <b>08 June 1995</b>	Accession Number <b>HB 11943</b>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of those designations in which a European or Australian Patent is sought or in any other states having equivalent provisions, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) <b>All</b>	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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Authorized officer		Authorized officer	